

# MYCOLOGIA

EDITOR  
FRED JAY SEAVER

Volume XX, 1928  
WITH 38 PLATES AND 14 FIGURES



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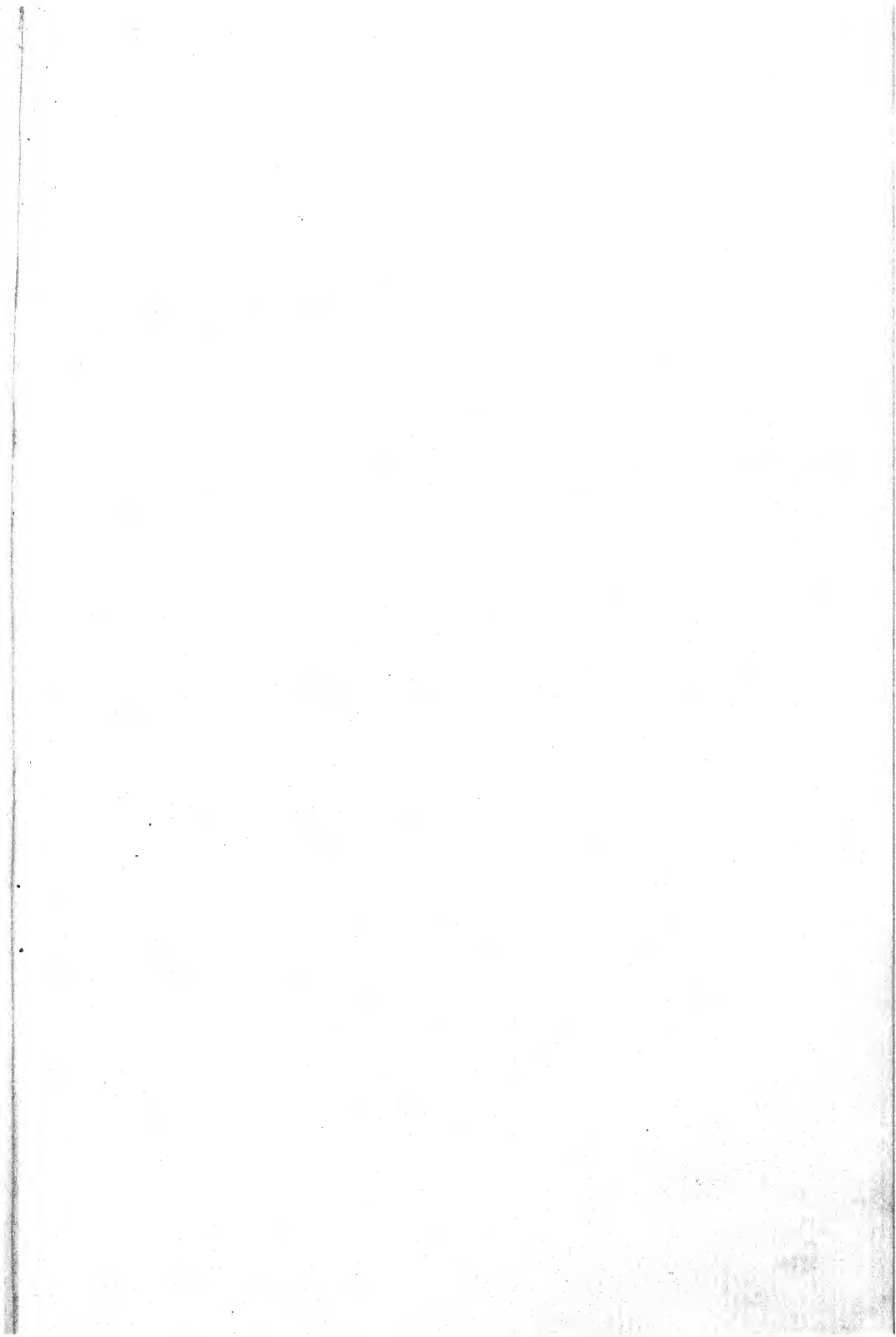
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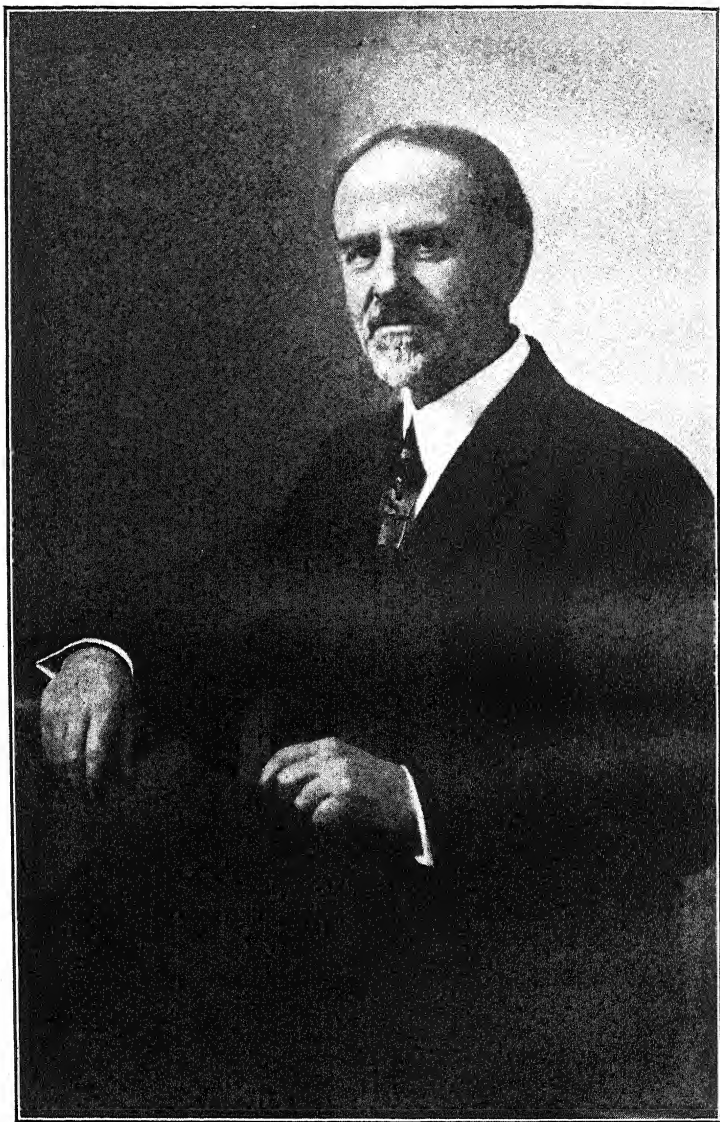
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BRUCE FINK

# MYCOLOGIA

VOL. XX

JAN.-FEB., 1928

No. I

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## BRUCE FINK, LICHENOLOGIST

ROBERT B. WYLIE

(WITH PLATE 1)

Dr. Bruce Fink, foremost lichenologist in this country, died July 10, 1927. Following a usual custom, he had gone to his laboratory early in the morning and an assistant there found him with his head resting on his arms, death having resulted from heart failure.

Born at Blackberry, Ill., Dec. 22, 1861, and obtaining degrees from Illinois, Harvard and Minnesota, Dr. Fink has been closely identified with the botanical work of this country for over thirty years. Beginning his teaching work at Upper Iowa University in 1892, he was made professor of botany at Grinnell College in 1903, and in 1906 accepted the headship of Botany at Miami University, which position he held until the end of his life.

Dr. Fink's research was mainly in the field of lichenology, and his activity as a scientist is attested by over one hundred titles, covering published notes, reviews, papers and monographs. Primarily a taxonomist, he contributed also to the biology of lichens with discussions of their classification, relationships and distribution. He had in preparation a manual of the lichens of the United States. Numerous papers dealt with fungi and particularly with the Ascomycetes. His botanical interests were broad and he followed with zest the marked development of the plant sciences as a whole which occurred during his scientific lifetime. Will any other period witness greater development and [MYCOLOGIA for November-December (19: 302-338) was issued November 1,

1927]

diversification than marked plant study and research from 1892 to 1927?

As a teacher, Professor Fink always carried a heavy class load and was greeted each year by large numbers of students. An unusual percentage of these were inspired to take advanced work in botany, and many of his students entered scientific work professionally.

Dr. Fink will be remembered by all who knew him as a man of genial personality and optimistic temperament. These qualities in combination with his modesty and generosity made him the friend of all classes. Of simple tastes, he loves his home and his laboratory and through these revealed a life of marked sincerity and high achievement.

UNIVERSITY OF IOWA,  
IOWA CITY, IOWA

# THE SEXUALITY AND ARRANGEMENT OF THE SPORES IN THE ASCUS OF NEUROSPORA SITOPHILA

MARGUERITE S. WILCOX

(WITH PLATE 2 AND 2 TEXT FIGURES)

Although considerable work has been done on the cytology of heterothallism in the Basidiomycetes, heretofore no one has investigated the sexual nature of particular spores in the asci of heterothallic Ascomycetes. Newton (23) determined experimentally the nature of the four spores as to their position on the basidium of *Coprinus Lagopus*. Betts (3) found that of seven single spore cultures from an ascus of *Ascobolus carbonarius* three were of one haplont and four of the other. Shear and Dodge (26) report that for the five different asci of *Neurospora crassa* examined four of the spores are of one sex and four of the other sex.

At a recent meeting of the Botanical Society of Washington, Dr. B. O. Dodge discussed the cytological basis for heterothallism in species of the *Monilia sitophila* group. He showed that while segregation of the factors for sexuality probably takes place in the first division in the four-spored homothallic species, *Neurospora tetrasperma* (26), it could take place in either of the other two divisions with the same effect. As a result of his studies he thought, however, that the differentiation of the sex character in the eight-spored heterothallic species *N. sitophila* (26) probably takes place in the second division, and that the spores in the ascus would alternate, two of one sex and two of the other. At his suggestion, and under his direction, the writer has undertaken to determine by means of cultures and a study of nuclear behavior the point of segregation of the sex factors in *N. sitophila*, and the sexual nature of each of the eight spores according to its position in the ascus.

In the basidium the orientation of the spindles during the mitoses and the position of the four daughter nuclei might not, owing to the particular features of the basidium, be any criterion

TABLE I

Fungus	Author	1st mitosis	2d mitosis	3d mitosis
<i>Peziza vesiculosa</i> . . . . .	Gjurasin (12)	long.	long. or obl.	trans.
<i>Peziza Stevensoniana</i> . . .	Harper (14)	long.	long.	
<i>Lachnea scutellata</i> . . . . .	Harper (15)	long.	long. or obl.	trans. or long.
<i>Pyronema confluens</i> . . . .	Harper (16)	trans.	long. or conj.	trans.*
<i>Galactinia succosa</i> . . . . .	Maire (22)	long.	long.	trans. or obl.
<i>Morchella esculenta</i> . . . .	Maire (22)	obl.	long.* or obl.	trans. or obl.
<i>Pustularia vesiculosa</i> . . .	Maire (22)	long.	long.	trans. or obl.
<i>Rhytisma acerinum</i> . . . .	Maire (22)	long.	—	trans.* or obl.
<i>Hydnobolites</i> sp. . . . .	Faull (7)	eccentric	periph- eral	parallel
<i>Neotiella albocincta</i> . . . .	Faull (7)	variable	variable	variable
<i>Sordaria fimicola</i> . . . . .	Faull (7)	long.*	long.*	trans. or obl.
<i>Phyllactinia corylea</i> . . . .	Harper (17)	long.	variable	variable
<i>Humaria rutilans</i> . . . . .	Fraser (9)	long.	obl.	trans. or obl.
<i>Otidea aurantia</i> . . . . .	Fraser & Welsford (11)	long. or obl.	long.	variable
<i>Peziza vesiculosa</i> . . . . .	Fraser & Welsford (11)	long.	long.	trans. or obl.
<i>Ascobolus furfuraceus</i> . .	Fraser & Brooks (10)	long. or obl.	long.	trans. or obl.
<i>Humaria granulata</i> . . . .	Fraser & Brooks (10)	—	trans.	trans.
<i>Lachnea stercorea</i> . . . . .	Fraser & Brooks (10)	obl.	trans. or obl.	trans.
<i>Geoglossum glabrum</i> . . . .	Jolivette (18)	long.	long.*	obl.
<i>Galactinia succosa</i> . . . . .	Guilliermond (13)	long.	long.	variable
<i>Pustularia vesiculosa</i> . . .	Guilliermond (13)	—	long.	trans.
<i>Pyronema confluens</i> . . . .	Claussen (4)	variable	variable	trans. or obl.
<i>Laboulbenia chaetophora</i>	Fauli (8)	long.	long.	long.
<i>Collema pulposum</i> . . . . .	Bachmann (1)	long.	—	long.
<i>Verpa bohemica</i> . . . . .	Komarnitzky (20)	long.	trans. or obl.	trans.
<i>Ascobolus immersus</i> . . . .	Ramlow (24)	trans.	trans. or obl.	long.
<i>Philocopra coeruleotecta</i>	Sax (25)	trans.	—	long.
<i>Pustularia bolarioides</i> . .	Bagchee (2)	long.	long. or obl.	trans. or obl.
<i>Ophiobolus graminis</i> . . .	Jones (19)	long.	long. obl. or conj.	variable
<i>Neurospora tetrasperma</i>	Dodge (6)	long.	long. obl. or conj.	trans. or obl.

\* Not figured, but orientation described in text.



for determination of the sexual nature of each of the four spores. There can be, however, no question that in the heterothallic Ascomycetes, especially those with long slender asci, the position of the daughter nuclei and orientation of spindles in the three mitoses do furnish, when taken in connection with the results of culture studies, a very reliable basis for determining when segregation of sex factors takes place.

As regards its orientation with respect to the long axis of the ascus, a spindle has been referred to as longitudinal, transverse or oblique. When the orientation is in any plane from longitudinal to transverse, the positions may be described as variable. Divisions in an ascus in which the spindles extend in the same direction and lie close together are referred to as conjugate, without implying that they serve the purpose usually attributed to conjugate division in the rusts. In table I are brought together chronologically statements of previous authors bearing on the position and orientation in the divisions of the nuclei of the asci in various species of Ascomycetes.

It will be seen from the table that in comparatively long asci the spindles of the first division are most frequently longitudinally orientated. The spindles of the second mitosis are also longitudinal, but less constantly so; while the spindles of the third mitosis are commonly transversely placed. Several exceptions in each case occur, however, which may or may not have some significance in connection with the segregation of particular factors.

Several investigators have worked out the cytology of the Basidiomycetes and find the spindles also variously placed. Levine (21), for example, states that the long axis of the spindles in both divisions is commonly transverse to the long axis of the basidium. The spindles may also lie in a position parallel to the long axis of the basidium.

#### CYTOLOGY OF THE ASCUS OF *Neurospora sitophila*

The material for cytological work was obtained from cultures made by inoculating corn meal agar test tubes with mycelia or conidia of the two reciprocal haplonts, *A* and *B* (26). In a week or ten days small pieces of agar-bearing perithecia were fixed in

Flemming's weaker solution. Sections were cut five microns thick and stained with the triple stain.

The ascus of *Neurospora sitophila* is long and narrow, such as is figured by Fraser & Welsford (11) for *Peziza vesiculosa*. The spindle of the first division lies in the long axis of the ascus, or nearly so, as is quite regularly the case in such long narrow asci.

After the first division is completed the reorganized daughter nuclei move some distance apart (PLATE 2, A). This drawing apart of the daughter nuclei is figured by Harper (17) in *Phyllactinia* and in *Peziza vesiculosa* by Fraser and Welsford (11). The second mitosis then takes place and the spindles are correspondingly some distance apart. They lie in the long axis of the ascus (PLATE 2, B).

The four resulting nuclei are somewhat bottle-shaped and have two long narrow antler-like structures extending from the neck (PLATE 2, C). The two nuclei in each half of the ascus are sister nuclei, symmetrically oriented. In every section in which this stage was found the horns of the first and third nuclei point upward while those of the second and fourth point downward. Aside from similar appendages described by Dodge (6) for *Neurospora tetrasperma* the writer has found no reference to anything resembling such structures in the literature on the fungi. In *N. sitophila* they are somewhat longer and more definitely developed organs than are those of *N. tetrasperma*. In the latter species, according to Dodge, frequently after the second division there must occur such a change of position of the pair of daughter nuclei as to insure that two non-sister nuclei come to lie in each end of the ascus. No such shifting occurs in *N. sitophila*.

The spindles of the third division are transverse and are placed some distance apart. At this stage the spore plasm of the ascus is divided into four parts, the regions lying between the spindles being filled with large vacuoles (PLATE 2, D). This would seem to preclude the possibility of any material change in the position either of the spindles during the third mitosis or of the pairs of daughter nuclei resulting from this division.

The stages between reorganization of the eight nuclei and spore delimitation are not figured. The astral rays do not show very

distinctly in preparations. Each nucleus is, however, drawn out into a long curved beak from which develop very fine rays which are to take part in cutting out of spores. Opposite the nuclear beak in each spore (PLATE 2, E) are developed numerous large vacuoles. The processes are essentially the same as described for the small spores of *N. tetrasperma* (6) where only one nucleus is included in each spore. Normally in *N. tetrasperma* each ascus contains four spores, two of the eight nuclei in the ascus being included in each spore. At spore delimitation these two nuclei lie side by side and coöperate in the process.

Shear and Dodge (26) have shown that each normal spore contains all the elements necessary for sexual reproduction so that the spore is ordinarily homothallic. In a later paper Dodge (6) has shown that when an ascus has more than four spores, the small spores are not totipotent and contain only one nucleus. Sexual reproduction can only be brought about in such cases by mating reciprocal haplonts obtained by germinating the small spores. In the normal or two-nucleate spores the nuclei are non-sisters, being developed at similar poles of adjacent spindles of the third division.

*N. sitophila* is heterothallic. Culture work to be described later in this paper shows that of the spores in one end of the ascus the upper two are of one sex and the next two of the opposite sex. The cytology of the ascus of this species just described shows how this comes about. The two spores uppermost in the ascus, for example, must contain nuclei which have developed at the poles of the upper spindle. The evidence presented all goes to show that segregation of the sex factors can not occur in either the third or first divisions. If segregation were to take place in the third division, the spores would alternate in the ascus, male and female. If segregation took place in the first division when reduction is presumed to occur, then all four spores in one end of the ascus would be of the same sex, which is certainly not the case in *N. sitophila*.

In such long and narrow asci there is little possibility of spores shifting positions with reference to each other, as might occur in a broad ascus of the type of *N. tetrasperma* or of powdery mildews. Still, there can be no question that the spores do readjust them-

selves by rotating as they elongate after being fully delimited. This is clearly shown in plate 2, *E*, where the nuclei of the upper four spores all point practically in the same direction while the nuclei of the lower four spores tend to point in the opposite direction. The careful study of two sections of this ascus indicates that the beaks of the nuclei are in reality arranged along a half spiral.

A later stage in the delimitation of the spores showing retraction of the nucleus is figured (PLATE 2, *F*). In this particular ascus all the spores were pointed the same way, showing clearly that considerable movement and twisting of the spores occur after they are cut out. The curved end of the beak which stains heavily still shows plainly long after the nucleus has become detached. While each spore is at first uninucleate, a further division takes place similar to those figured by Maire (22) in *Morchella*, Faull (7) in *Sordaria*, and Dodge (6) in *Neurospora tetrasperma*, so that each spore of *N. sitophila* at maturity contains two sister nuclei (PLATE 2, *H*).

#### CULTURE STUDIES

Ascospore material for this study of *Neurospora sitophila* originally came from Arlington, Va., and Japan (26). The perithecia used were grown in culture made by transferring to corn meal agar bits of mycelia or conidia from cultures having mycelia of both sexes. It was found that the spores would not germinate if isolated from the ascus before they have become fully colored. If, on the other hand, the ascus is too old, the wall is apt to burst before its spores can be isolated in order.

The point of an ordinary dissecting needle was too large for isolating the spores from the ascus, although an expensive micro-manipulator is not at all essential. The finest sewing needles that could be purchased were found to be quite satisfactory.

Five or six perithecia which are just beginning to discharge their first spores were crushed out in a drop of sterile water on a slide. After a number of mature asci had been separated from the crushed masses they were transferred with a part of the water to 4 per cent corn meal agar in petri dishes. Using a binocular microscope and a fine pointed needle, individual asci were isolated

on the surface of this hard agar. The spores could then be removed one by one from the ascus and pushed a few millimeters away where their relative positions in the ascus were recorded by scratching the number on the agar.

These ascospores do not ordinarily germinate at room temperature (26). In this they are like spores of certain species of *Ascobolus* which must be heated before they will germinate. The method described by Dodge (5) has been found adequate for this work. Since *Neurospora sitophila* produces conidia in abundance, special precautions must be taken to avoid contamination of the single ascospore cultures. After isolating the ascospores it is advisable to allow the petri dish to stand for several hours so the conidia may begin to germinate. The germinating conidia can then be killed by the heating necessary to germinate the ascospores (26).

The plates containing the isolated ascospores were placed in an oven at room temperature and slowly raised to about 90° C. temperature. This usually took from fifteen to twenty minutes. In from five to twelve hours after heating, depending on temperature of room where cultures were kept, some spores were found to have germinated. Each germinating spore was then carefully removed on a bit of agar and transferred directly to corn meal agar in a test tube.

In about three days or as soon as enough conidia had formed in these cultures, masses of conidia of each haplont were transferred to separate tubes of melted agar. To avoid possibility of contamination or mixture of spores of the various haplonts, different culture rooms were used for making the transfers. After the agar containing spore suspensions had hardened, transfers could be made without the danger of contaminations such as would occur if dry conidia were transferred directly. All possible combinations of the haplonts from each ascus were then made. Perithecia usually begin to develop within eight or ten days, but as they at first resemble bodies which soon appear in the tubes containing single haplonts, it is necessary to wait two or three weeks before results can be determined definitely.

As might be expected, in the majority of the trials the writer failed to obtain germination of all eight spores in the ascus.

Unlike the spores of *Ascobolus carbonarius* which will germinate before they are mature and while still colorless or half grown (5) the spores of *Neurospora sitophila* must reach full maturity before germination will occur.

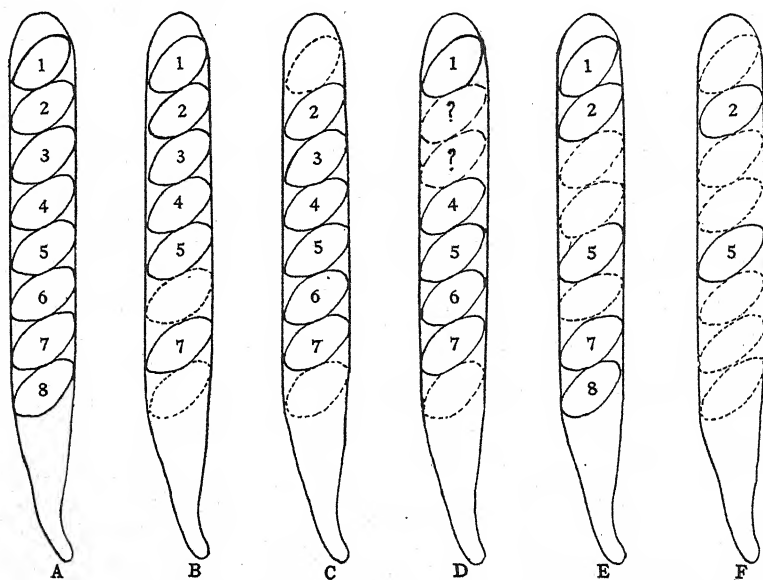


FIG. 1. Diagram of asci showing number and position of spores germinated after isolating from ascus. Germinated spores are shown in solid line

In only one instance was full germination obtained, but in five other cases the position of the spores that did germinate was such that it could be determined with practical certainty what the sexuality of the missing spores originally was. The positions of the spores from which haplont mycelia were obtained are shown in text figure 1. The spores are numbered from the tip of the ascus downward.

As noted above, in only one instance (A) did all eight of the spores germinate. In three cases (B, C, D) six of the spores germinated; in one case (E) five spores developed mycelia and in one trial (F) only two spores grew. At this point it may be stated that the terms "sex" and "sexuality" are used without knowing whether or not a monosporous mycelium of *N. sitophila* produces functional oögonia or antheridia. A monosporous

mycelium is referred to as "haplont." The results of mating the eight different haplonts obtained by germinating the eight spores in the ascus represented by *A* in text figure 1 are given in table II. Failure to produce perithecia is indicated by a minus sign (—), a positive sexual reaction resulting in the formation of normal perithecia is indicated by the plus sign (+).

TABLE II

RESULTS OF GROWING TOGETHER IN DUPLICATE IN ALL POSSIBLE COMBINATIONS THE EIGHT HAPLONT MYCELIA OBTAINED BY GERMINATING THE EIGHT ASCOSPORES FROM A SINGLE ASCUS OF *Neurospora sitophila*

	1	2	3	4	5	6	7	8
1.	—	—	+	+	+	+	—	—
2.	—	—	+	+	+	+	—	—
3.	+	+	—	—	—	—	+	+
4.	+	+	—	—	—	—	+	+
5.	+	+	—	—	—	—	+	+
6.	+	+	—	—	—	—	+	+
7.	—	—	+	+	+	+	—	—
8.	—	—	+	+	+	+	—	—

Mycelia from spores Nos. 1, 2, 7 and 8 in this ascus produce no perithecia when grown in combination with each other. They must, therefore, have like sex factors. Mycelia from spores Nos. 3, 4, 5 and 6 likewise produce negative results when grown with each other and are, then, like haplonts as to their sexuality. It will be seen, however, that when the haplont from spore No. 1, 2, 7 or 8 is grown with the haplont from spore No. 3, 4, 5 or 6, a positive sexual reaction occurs which is indicated by the formation of perithecia.

The particular sex of the haplont in no case has as yet been determined. Shear and Dodge (26) have referred to haplonts of this species which were reciprocal as to their sexuality merely as haplonts *A* and *B*. The writer has determined the sexuality of the eight haplonts under discussion by mating haplonts No. 2 and 5 with the known haplonts *A* and *B* obtained from those authors.

The results obtained show the spores 1, 2, 7 and 8 contain what for convenience may be called the *B* factors for sexuality while spores 3, 4, 5 and 6 contain the *A* factors for sexuality.



These results are shown diagrammatically in text figure 2, where the spores which developed haplont *A* are shown in white and haplont *B* in black.

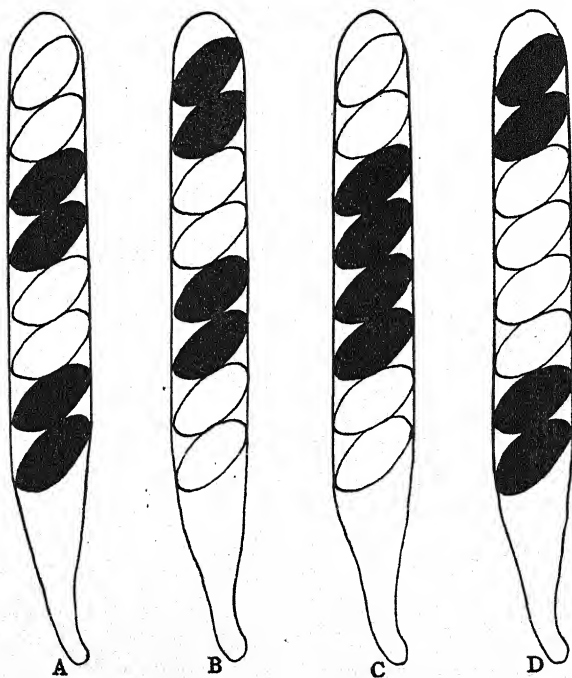


FIG. 2. Diagram showing the four possible arrangements of spores in the ascus according to sex as determined by cultural results. White spores are haplont *A*, blackened spores are haplont *B*.

In ascus *B*, text figure 1, the 6th and 8th spores did not germinate. Judging from what is known from the writer's cytological studies of *N. sitophila* previously discussed in this paper, and from the results obtained by the pairing of like haplonts as has just been shown where the sex nature of each spore in the ascus was determined experimentally, spore No. 6 should carry the same sex factors as spore No. 5 and spore No. 8 should bear the same factors for sexuality as spore No. 7. The results obtained after mating spores from this ascus with known haplonts *A* and *B* (26) are shown in text figure 2-A. In this ascus the sexes of the spores alternate in pairs, the first pair carrying the *A* sex factors, the second pair carrying the *B* sex factors, and so on.



In trial No. 3 (TEXT FIG. 1-C) spores Nos. 1 and 8 were missing. On the basis discussed above one can assume that spore No. 1 is like spore No. 2, and that spore No. 8 is like spore No. 7. Text figure 2-B will then represent the picture showing the results actually obtained by growing six of the eight haplonts in all possible combinations as completed by the addition of the missing haplonts, Nos. 1 and 8. Here, too, the sexes of the spores alternate in pairs, the first and third pairs in this instance carrying the *B* sex factors and the second and fourth pairs bearing the *A* factors for sexuality, the reverse of the condition represented in text figure 2-A.

In ascus "*D*" (TEXT FIG. 1) spores 1, 3(?), 4, 5, 6 and 7 developed mycelia. The results of the combinations made show that spores 1, 5 and 6 carried the *A* factors for sexuality and spores 4 and 7 the *B* sex factors. In isolating the spores from this ascus the writer was not positive of the order of the 2d and 3d spores, and this was borne out by the results of the culture. According to the results of the pairing, the haplonts in the upper end of the ascus alternated, and it is assumed that the spore originally labeled No. 3 was in reality the second spore in the ascus. As previously discussed, all evidence goes to show that like haplonts arrange themselves in pairs along the length of the ascus. Supplying the missing spores on that basis, the picture figure 2-A, formed by the arrangement of the spores in this ascus, is the same as that for ascus *B*, discussed previously. In general there is no tendency to shift positions after the spore has been delimited. The fact that the ascus is long and narrow and the third division spindles are placed far apart and transversely oriented is an assurance against any shifting or displacement of the spores. If, however, the eight spores of a large number of asci were germinated, it would be indeed strange if an exception to the general order would not be found, so that an alternation of sexes in one end of the ascus might result.

The results of making all possible combinations in culture of the five haplonts which were obtained by germinating the spores indicated in text figure 1-E forms the picture *B* of text figure 2 as completed by inserting the missing haplonts on the basis discussed above. The arrangement of the spore haplonts in

this ascus is the same as that of ascus *C* (TEXT FIG. 1). Spores 1, 2 and 5 are haplont *B* and spores 7 and 8 haplont *A*.

It might be thought that when only two spores from an ascus germinate, such results should be disregarded as of no consequence. Even such meager results are helpful in arriving at a conclusion as to the constancy of the location in the ascus of "male" and "female" spores. In ascus "*F*" (TEXT FIG. 1) only the second and fifth spores germinated, and when combined with each other and with known haplonts, spore No. 2 proved to be haplont *A* and spore No. 5 haplont *B*. From what is known of the pairing of haplonts, it is assumed that spore No. 1 bears the same sex factors as spore No. 2, namely "*A*," and that spore No. 6, like spore No. 5, carries the "*B*" factors for sexuality. The evidence afforded by a knowledge of the nuclear behavior in the ascus, supplemented by the results of the culture work just presented, supports the claim previously set forth (p. 7) that segregation of sex factors in this species must take place in the second division in the ascus. Therefore, if the first two spores of this ascus are haplonts *A*, spores 3 and 4 must be of the opposite sex, or haplonts *B*. If spores 5 and 6 are haplonts *B*, the spores 7 and 8 must contain the *A* sex factors, making the picture shown in "*C*" of text figure 2.

Of the six asci of *N. sitophila* described above the spore haplonts in two of the asci form the picture *AA*, *BB*, *AA*, *BB*; two asci form the picture *BB*, *AA*, *BB*, *AA*; and one ascus each makes the picture *AA*, *BB*, *BB*, *AA* and *BB*, *AA*, *AA*, *BB*, respectively. In every case it will be seen the spores arrange themselves in pairs with both sexes in each end of the ascus. This is explained by a study of the cytology of the species. The ascus is long and narrow and the spindle of the first mitosis is longitudinally placed. The resulting daughter nuclei move some distance apart and the spindles of the second division are also longitudinal. The four resting nuclei are distributed at equal distances along the length of the ascus and, upon dividing, the spindles are transversely oriented. Between the spindles the sporeplasm is filled with large vacuoles, which would tend to prevent any material change in the position either of the spindles of the third division or of the pairs of resulting daughter nuclei.

The two upper spores, then, must contain the nuclei which developed at the poles of the upper spindle and are, therefore, sister nuclei, the spores carrying like factors for sexuality.

Very little work has been done to ascertain the location of haplonts in *N. crassa*. Three spores, the location of which was determined in each case, from each of two different asci, were germinated. The results obtained by growing these haplonts in all possible combinations and with known haplonts *A* and *B* of this species further uphold the theory that the segregation of the sex factors takes place in the second mitosis.

#### SUMMARY

In *Neurospora sitophila*, the ascus is long and narrow and the spindle of the first mitosis is longitudinally placed. The resulting daughter nuclei come to rest some distance apart, one above the other in the ascus. The second division spindles are also longitudinal.

The four resting nuclei are bottle-shaped and have two long horn-like appendages protruding from the neck. The spindles of the third division are transverse and are widely separated.

Spores of known position were isolated from six asci of *Neurospora sitophila* and every possible combination made in cultures. It was found that the spore haplonts may be arranged in the ascus in either one of the four following combinations, *AA, BB, AA, BB*; *BB, AA, BB, AA*; *AA, BB, BB, AA*; or *BB, AA, AA, BB*, where *A* and *B* are reciprocal as to their sexuality.

Since the spores carrying like factors for sexuality arrange themselves in pairs along the length of the ascus, the segregation of the sex factors must take place in the second mitosis. Had this factor been segregated in the first division, the four spores at one end of the ascus would be of one sex and the four spores at the other end of the opposite sex. Should this segregation take place in the third mitosis, however, the spores of opposite sex would alternate throughout the length of the ascus, which would be contrary to the experimental results.

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## EXPLANATION OF PLATE 2

Fig. A. Resting daughter nuclei, two-nucleate stage. Nuclei some distance apart.

Fig. B. Second division in metaphase stage. The spindles of this mitosis are longitudinal.

Fig. C. Four-nucleate stage. The second nucleus counting from above lay at a slightly higher focus in the section.

Fig. D. Third division, anaphase stages showing the even distribution and transverse orientation of spindles. The lower spindle is in the same ascus but in another section. The sporeplasm is divided into four regions by large vacuoles.

Fig. E. Delimitation of spores. The portions lying in a higher focus in the section are more heavily shaded. The nuclei are much elongated and end in a curved beak.

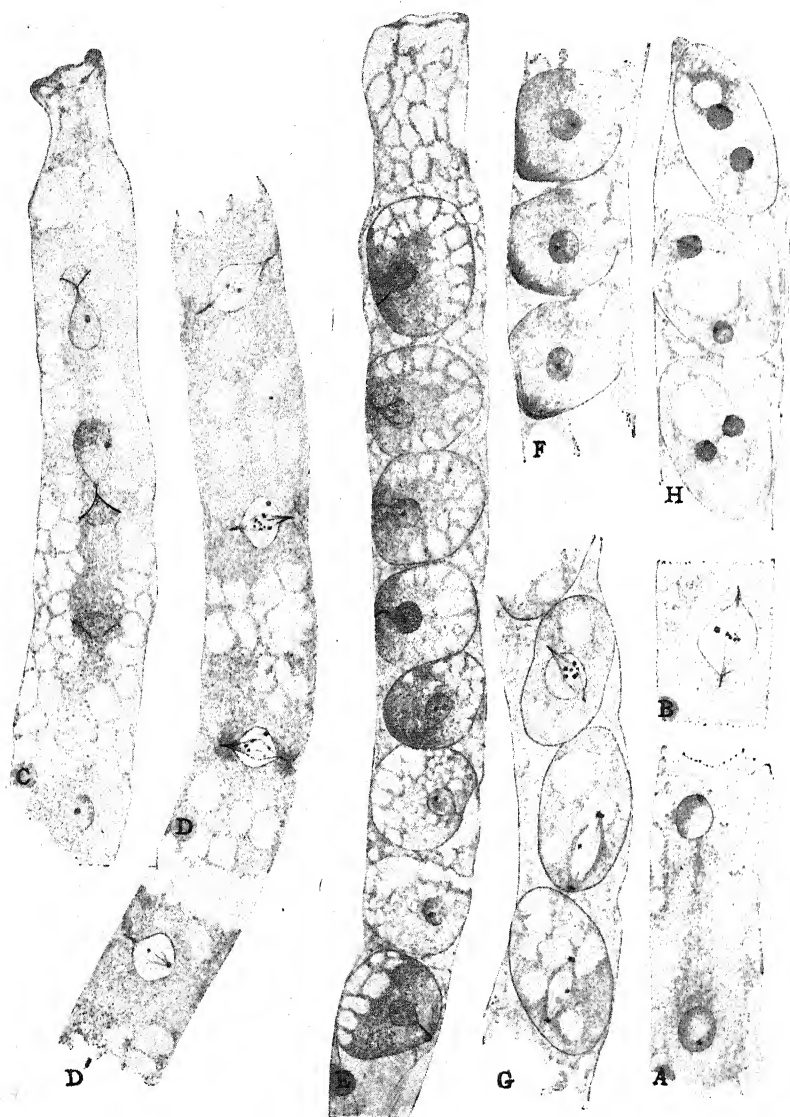
Fig. F. Later stage in delimitation of spores showing retraction of nuclei. The hooked end of such nuclei still visible at the apex of each spore.

Fig. G. Karyokinesis in spores.

Fig. H. Mature spores, showing two nuclei.

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## SPORE FORMATION IN ASCI WITH FEWER THAN EIGHT SPORES

B. O. DODGE

The fusion nucleus in the ascus of the higher ascomycetes regularly divides three times in succession. In case more than eight spores are to be developed in the ascus, the eight nuclei undergo further simultaneous divisions so that 16, 32, or more nuclei are formed before spore delimitation. The writer has counted 1024 spores in certain asci in *Thelebolus stercoreus*. There must have been at least 1024 nuclei resulting from the seven additional divisions in the ascus before spore formation. Spore formation in such asci has been worked out by Overton (6), Sax (7), and others. In case an ascus should develop fewer than eight spores, as in *Phyllactinia corylea*, for example, which very commonly develops two spores, Harper (4) has shown that six of the nuclei degenerate so that each spore contains only one nucleus at its origin. Komarnitzky (5) further confirmed this doctrine of degeneration of nuclei in connection with *Verpa bohemica* (*Morchella bispora*) the ascus of which has only two spores. Komarnitzky's figures are not wholly conclusive; nevertheless, it would seem from his discussion that six of the nuclei degenerate, only two taking part in spore formation, one for each spore. Faull (3) has studied two species of *Laboulbenia*. Most species of this order develop only four spores in an ascus. The four spindles of the third division are longitudinal. The nucleus formed at the upper end of each spindle migrates to the top of the ascus and degenerates. One of the four nuclei from the lower ends of the spindles then is included in each spore as it is cut out.

Wolf was the first to report the inclusion of two nuclei regularly at the formation of each spore in an ascus. The ascus of *Podospora anserina* usually forms only four spores. Wolf (9) says that two of the eight nuclei in the ascus enter each spore. Inas-



much as he failed to furnish all of the details of nuclear behavior leading up to spore formation, and as this process was contrary to what had heretofore been reported in other species, his paper has not perhaps received the attention which it deserves. Two or three other cases where two or more nuclei take part in spore formation have been mentioned incidentally merely as abnormalities. The writer (2) has recently studied spore formation in the four-spored ascus of *Neurospora tetrasperma* Shear and Dodge (8). It has been found that the eight nuclei of the ascus come together in pairs, side by side, so that two nuclei, one of each sex, take part in the formation of each spore. Occasionally an ascus may develop five spores. In such case two of the spores will each contain only one nucleus. Whereas *Neurospora sitophila* is heterothallic, mycelia derived from the binucleate spores of *N. tetrasperma* are homothallic. On the other hand, a mycelium from a uninucleate spore of this species does not develop perithecia when grown alone. When two such mycelia of opposite sex are grown together on a suitable medium, ascocarps with four-spored asci are produced. Should an ascus develop only two large spores, each one will contain four of the eight original nuclei. A mycelium from such spores is totipotent.

A number of years ago while working on leaf forms of *Gymnosporangium* on *Chamaecyparis* in the pine barrens of New Jersey, the writer found a tree at Lakehurst which was badly infected with what appeared to be a new species of *Keithia*. A preliminary study of nuclear behavior in the ascus convinced the writer at the time that each of the two spores in the ascus originally contains four nuclei at the time the spore is cut out. As the fixation of this material was not wholly satisfactory for cytological study so that the details of nuclear behavior at various stages leading up to spore formation could not be determined readily, this material was turned over to Dr. J. F. Adams (1), who continued the study on additional material and later published a short account of the parasitism and general morphology of the fungus and described it as a new species, *Keithia Chamaecyparissi*.

The writer has recently reexamined his old preparations as well as the slides kindly loaned him by Dr. Adams. It would appear

that in this species of *Keithia* each spore contains originally four nuclei. The four nuclei cooperating in spore formation seem to be arranged more or less equatorially in the young spore, the beaks pointing outward. Later the nuclei become distributed irregularly and finally crowd toward the upper or tip end of the spore. Whether or not the four nuclei undergo further division just before the septum is laid down, cutting off a very small segment at the upper end of the spore, cannot be determined from this material. In any event, the small segment contains at least two nuclei. Additional material has been recently obtained from Lakehurst and it is hoped that further information will soon be available. The point to be emphasized is that here we have another species of ascomycete with fewer than eight spores and in the delimitation of the ascospores of which more than one nucleus is regularly concerned.

It is well known that where a species commonly develops eight spores in an ascus, occasionally one or more of the spores for some reason will fail to reach full maturity, or abort after it has been delimited. The powdery mildews which develop fewer than eight spores are in still another category since certain nuclei degenerate without first cutting out spores. *Keithia thujina* also develops only two spores, and there are several other well-known species of ascomycetes which usually mature fewer than eight spores in an ascus. The question arises, is the failure in a particular case to mature eight spores due (1) to an abortion of certain spores after their delimitation, (2) do certain of the nuclei degenerate, or (3) do two or more nuclei cooperate in the processes of spore delimitation? In *Neurospora tetrasperma* the inclusion of two nuclei in each spore serves a very useful purpose since the factors for sex, as it may be called for convenience, are segregated out before the spores are delimited. The distribution of the nuclei in the ascus and the orientation of the spindles is such that two nuclei of opposite "sex" can readily cooperate in cutting out a spore. On rare occasions *Keithia* develops more than two spores. Sections of an ascocarp show one ascus in which four spores were originally delimited. The largest spore contains three nuclei, two spores have two nuclei each, and the smallest spore only one. Perhaps such small spores lacking the full com-

plement of nuclei possess a different genetic constitution. May they not sometimes even be heterothallic?

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## NOTES ON THE PARASITOLOGY OF SCLEROTIUM ROLFSII

RUTH DAVIS PAINTIN

(WITH PLATES 3 AND 4 AND 1 TEXT FIGURE)

The many studies of *Sclerotium Rolfsii* Sacc. which have been made since it was first reported by Rolfs (3) in 1893 seem to indicate that the fungus is a weak parasite with no host preference. It has previously been recorded as parasitic on about one hundred and forty species and varieties of flowering plants, including such important crops as: beans, potatoes, cowpeas, peppers, melons, and tomatoes. It is the cause of considerable annual loss in corn, wheat, cotton, rice, and tobacco. *S. Rolfsii* also infects numerous ornamental plants, for example: *Campanula*, *Coreopsis*, *Chrysanthemum*, *Delphinium*, *Daphne*, *Dahlia*, carnation, sweetpea, and violet.

In general the losses from this disease are not severe, except in isolated fields. However, the percentage of damage seems to be gradually increasing according to the reports of the Plant Disease Survey, and its control is becoming a serious agricultural problem especially in the southeastern United States.

Although *S. Rolfsii* is especially prevalent in the southeastern United States, recent reports show isolated occurrences in Pennsylvania, Ohio, Illinois, Indiana, Nebraska, and California. It has also been reported from China, Australia, and numerous islands of the Pacific and Atlantic oceans. It has been found in Uganda, Africa, and in the Vorstenlanden. The writer isolated the fungus from a diseased potato purchased in Chicago, but shipped from Virginia.

### NEW HOSTS

A series of soil inoculation experiments was conducted in the green house using sclerotia from cultures isolated from Lima bean in Florida by G. F. Weber and from Potato in Texas by J. J. Taubenhau.

From these experiments the following named plants, not previously reported, have been determined by the writer as hosts:

<i>Begonia</i> sp.	<i>Melilotus alba</i> Desr.
<i>Brassica Rapa</i> L.	<i>Myriophyllum</i> sp.
<i>Brassica</i> sp. (var. Improved Ostrich Plume)	<i>Ornithogalum umbellatum</i> L.
<i>Bryophyllum pinnatum</i> Kurz.	<i>Oxalis</i> sp.
<i>Chenopodium</i> sp.	<i>Petroselinum hortense</i> Hoffm.
<i>Cichorium Endivia</i> L.	<i>Phaseolus multiflorus</i> Willd.
<i>Coleus Blumei</i> Benth.	<i>Pteris longifolia</i> L.
<i>Commelina</i> sp.	<i>Reseda odorata</i> L.
<i>Cucurbita Pepo</i> Baily (var. <i>ovifera</i> )	<i>Ricinus communis</i> L.
<i>Dolichos multiflorus</i> Torr. & Gray	<i>Salvia officinalis</i> L.
(var. Early Speckle)	<i>Thymus vulgaris</i> L.
<i>Echinochloa frumentacea</i> Link	<i>Triticum aestivum</i> L. (var. Marquis)
<i>Hordeum sativum</i> Jess. (beardless)	<i>Tropaeolum</i> sp.
<i>Impatiens Sultani</i> Hook.	<i>Vinga sinensis</i> Endl. (var. Clay)
	<i>Zea everta</i> Sturtev.

#### METHODS

Very little histological work has been reported in connection with this fungus. The only record of a stain used is contained in a brief note by Rolfs (3) who mentions picrocarmine.<sup>1</sup>

For the purpose of histological study host tissues were prepared as follows: The materials were killed in Flemming's solutions, weak or strong, or in chromacetic acid. The time allowed for the action of these various agents was about 24 hours. When Flemming's stronger solution was employed in preparing the material, mixture *A*, containing osmic acid, was used in combination with solution *B*, for one hour. For the remainder of the time the material was kept in solution *B* which lacked osmic acid. This practice was employed to prevent darkening of tissues. If bleaching was necessary, a mixture of hydrogen peroxide and 50 per cent alcohol solution was used. Paraffin sections were made 10 to 12  $\mu$  in thickness. Dickson's (1) differential stain, with some modifications, was found most satisfactory. The procedure was: magdala red, ten minutes; rapid rinse in 85 per cent alcohol; light green, one half to one minute; carbol turpentine or 100 per cent alcohol for clearing; mount in balsam. This process is differential, usually staining the fungus a reddish purple, while the parenchyma takes on a light

<sup>1</sup> B. B. Higgins' paper, Physiology and parasitism of *Sclerotium Rolfsii* Sacc. Phytopathology 17: 417-448. 1927, appeared after this paper was in proof.

green color. The xylem strands stain reddish, thereby not making a clear color contrast between the fungal hyphae and the lignified tissue.

#### PATHOLOGICAL HISTOLOGY OF THE HOST

Abrasion or wounding of the host is not necessary for infection. Penetration is accomplished either directly by piercing or by a dissolution of the cell wall. The fungus usually gains entrance into the plant by mass action (PLATE 3, FIG. 2; TEXT FIG. 1) a little below the surface of the soil. However, an exception to this was noted in the case of a single hypha which was observed to have entered through a hair cell into the cortex of the stem of cowpea. Also single, intercellular hyphae, which dissolved the middle lamellae, were observed to have passed through the walls



FIG. 1. Cross-section of the stem of cowpea showing the typical radial fans or mats of mycelium invading the cortex.  $\times 700$ . Camera lucida. *a*, hyphae constricted between cells.

into the cells. In general, however, the hyphae enter the epidermal cells in a mass, grow abundantly through the cortex both intercellularly and intracellularly and cause a very severe infection (PLATE 4). This observation differs from the statement of Edson and Shapovalov (2) that hyphae usually do not enter the tissue directly, but a disorganization takes place due to digestive enzymes.

The mycelium was observed in the stele of the crown of cowpea and velvet bean seedlings, where the hyphae travel up into the stems (PLATE 3, FIGS. 3, 4) and down into the roots of the plants. Frequently the fungus brings about a complete disintegration of the central cylinder. In the region of initial infection the cortex is usually most severely invaded. The hyphae either travel through the tissue from cell to cell, piercing (PLATE 4, FIG. 1a) or dissolving (PLATE 4, FIG. 2a) the cell wall, or they grow in the cell wall, dissolving the middle lamella (PLATE 3, FIG. 1) and often distending the walls with the mass of crowded hyphae. More often the hyphae pass through the cell wall without constriction (PLATE 4, FIG. 1b), as has been observed by Small (4), but they may be constricted (TEXT FIG. 1a). Hyphae in large fascicles may travel up and down a stem of cowpea, invading in a mass. The growing hyphae exert a considerable pressure as is shown by the frequent distending or bulging of the host cell walls when the tips of hyphae come in contact with them. In the final phase of severe infection all that remains of the host is a few disorganized fragments of the cell walls among the extensive mat of hyphae.

The writer wishes to express appreciation for advice and supervision of this study by Professor Alfred Povah; also to acknowledge the courtesy of Messrs. Taubenhaus, Whetzel, Weber, Edgerton and Moreland for sending cultures.

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#### EXPLANATION OF PLATES

##### PLATE 3

Fig. 1. Intercellular mycelium in the stem of cowpea showing vacuoles and nuclei or food bodies.  $\times 1500$ .

Fig. 2. A longi-section through a velvet bean stem, near the region of infection, showing the entrance of the fungus in a mass.  $\times 1300$ . Projection microscope.

Fig. 3. A longi-section of the stele of an infected cowpea stem.  $\times 1000$ . Camera lucida.

Fig. 4. A longi-section of the stele of an infected velvet bean stem.  $\times 1300$ . Camera lucida.

#### PLATE 4

Fig. 1. Cross-section of the cortex of an infected cowpea stem, near the crown.  $\times 1300$ . Projection microscope.

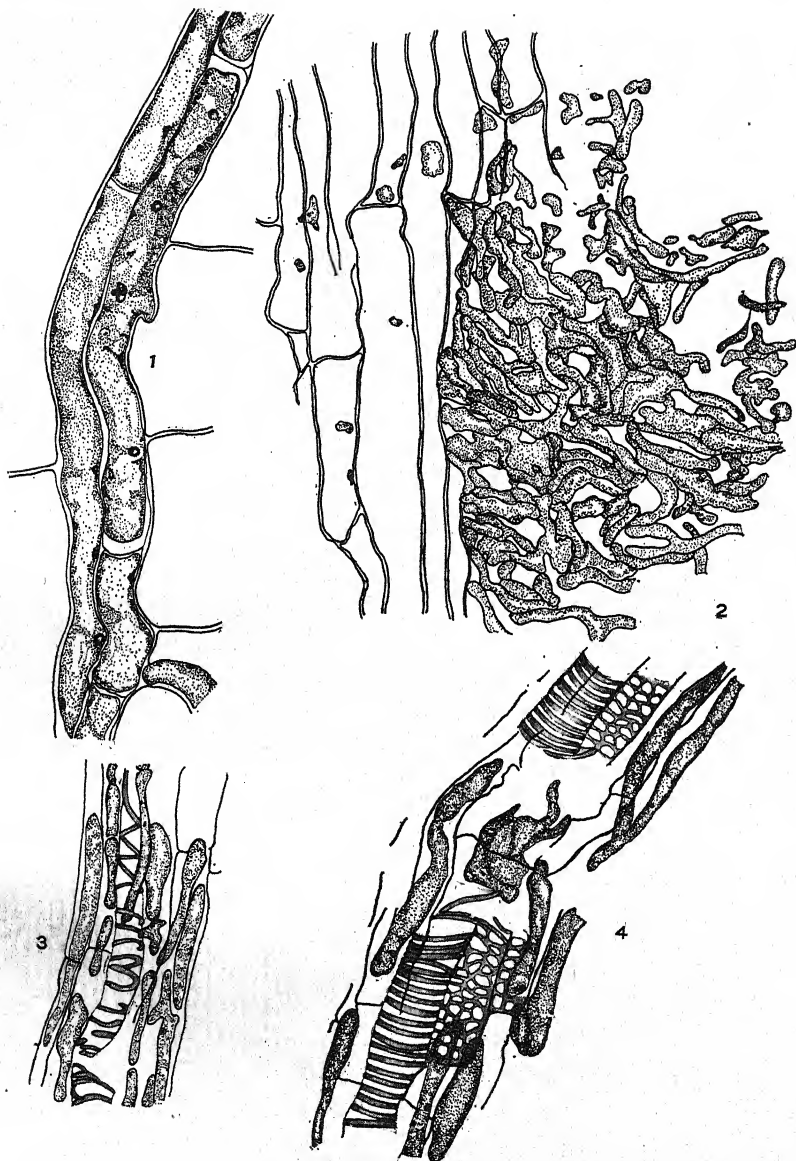
*a*, wall pierced by hyphae.

*b*, hyphae passing from cell to cell without constriction.

Fig. 2. Cross-section of the cortical region of a heavily infected velvet bean stem, showing the intercellular mycelium.  $\times 1300$ . Projection microscope. *a*, walls dissolved by hyphae.

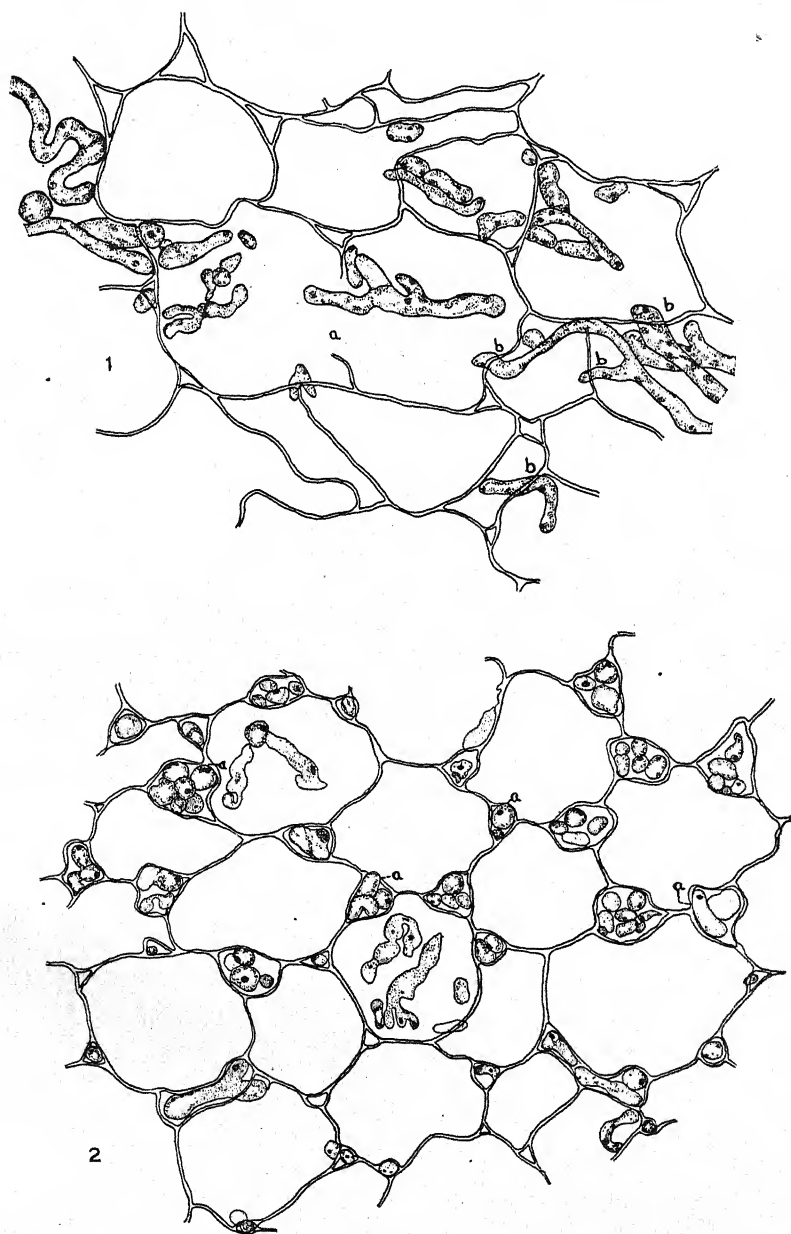
(Drawings reduced one-third in reproduction.)



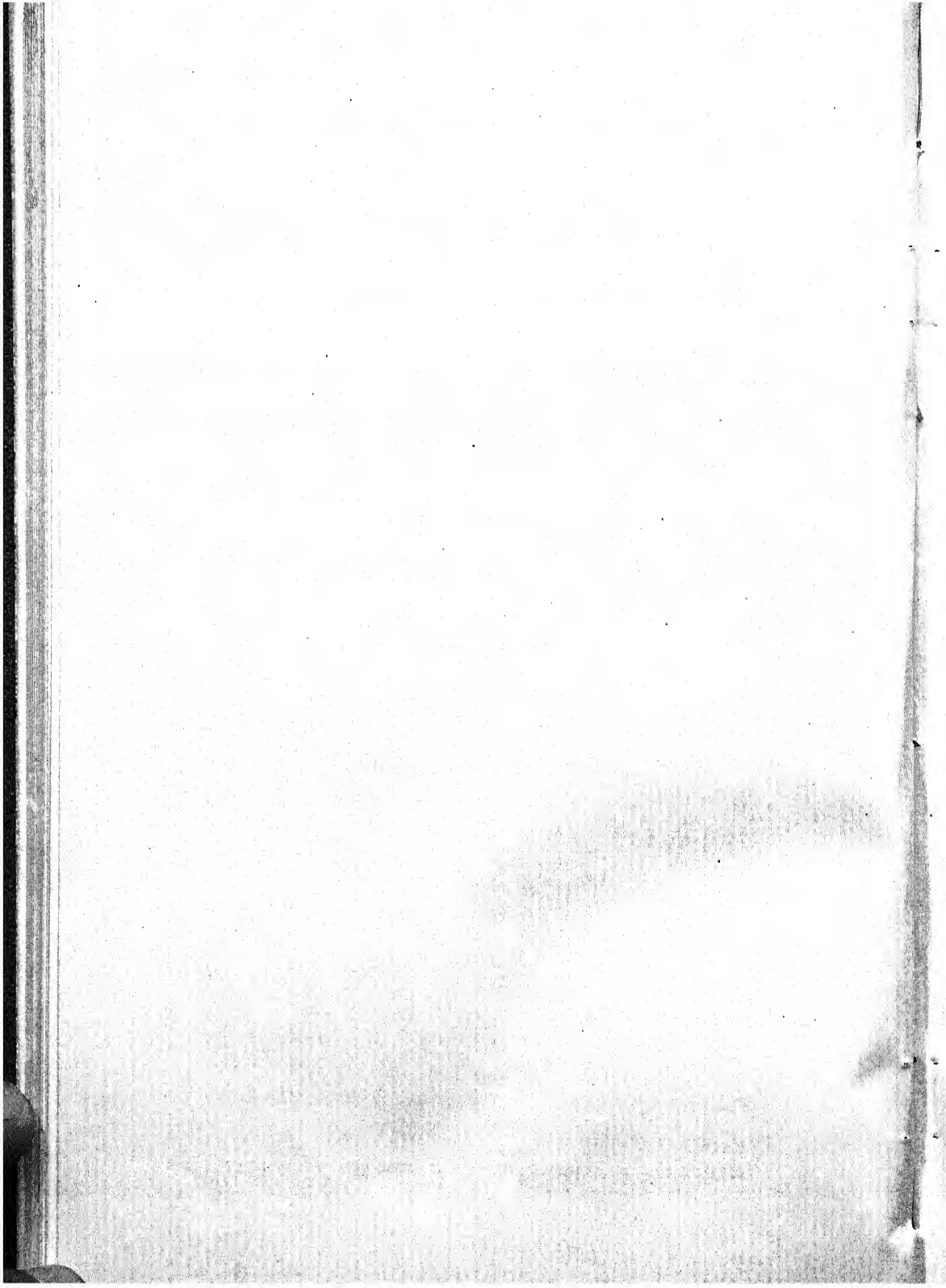


STEM SECTIONS OF VELVET BEAN AND COWPEA INFECTED WITH *SCLEROTIUM ROLFII*





CROSS-SECTION OF INFECTED STEMS OF COWPEA AND VELVET BEAN



## MYXOMYCETES FROM BRITISH GUIANA AND SURINAM

FRANK A. GILBERT

During the winter of 1923-24, Dr. David H. Linder, while a Sheldon Traveling Fellow from Harvard University, made a collection of fungi from British Guiana and Surinam. In this collection were a number of Myxomycetes which were turned over to the writer for identification and are now in the Farlow Herbarium. These Myxomycetes are not new, nor even extremely rare, in fact the majority are widely distributed throughout the world, but coming as they do from that part of South America where few collections of fungi have been made, their occurrence is of geographical importance and considerably extends the ranges of a number of species.

In this list, which follows, the locality, date, and collector's number are given under each species. Unfortunately, notes upon the character of the substratum were not taken in most cases.

CERATIOMYXA FRUTICULOSA (Muell.) Macbr.

Bartica, B. G.; December 1923; Linder 608.

CERATIOMYXA FRUTICULOSA (Muell.) Macbr. var. FLEXUOSA  
Lister.

Bartica, B. G.; December 1923; Linder 608a.

Tumatumari, B. G.; September 15, 1923; Linder 216.

PHYSARUM BOGORIENSE Racib.

Botanic garden, Paramaribo, Surinam; November 8, 1923;  
Linder 368.

Plantation Vryheid, B. G.; February 1, 1924; Linder 878.

PHYSARUM POLYCEPHALUM Schw.

Plantation Vryheid, B. G.; February 1, 1924; Linder 919.

PHYSARUM TENERUM Rex.

Bartica, B. G.; January 20, 1924; Linder 733.

*TRICHAMPHORA PEZIZOIDEA* Jungh.

Botanic Garden, Surinam; November 1923; Linder 321.

*PHYSARELLA OBLONGA* (Berk. & Curt.) Morg.

Plantation Vryheid, B. G.; February 28, 1924; Linder 1001.

*DIDYMIUM NIGRIPES* (Link) Fr.

Bartica, B. G.; December 19, 1923; Linder 491.

*COMATRICHA TYPHOIDES* (Bull.) Rost.

Plantation Vryheid, B. G.; February 16, 1924; Linder 962.

*TUBIFERA STIPITATA* (Berk. & Rav.) Macbr.

In wet forest, near botanic garden, Surinam; November 4, 1923; Linder 317.

*LYCOGALA EPIDENDRUM* (Buxb.) Fr. var. *EXIGUUM* Lister.

Bartica, B. G.; January 19, 1924; Linder 784.

*ARCYRIA CINEREA* (Bull.) Pers.

Botanic garden, Paramaribo, Surinam; November 7, 1923; Linder 356.

*ARCYRIA CINEREA* (Bull.) Pers. var. *DIGITATA* G. Lister.

Decayed log, Tumatumari, B. G.; September 15, 1923; Linder 217.

*ARCYRIA DENUDATA* (L.) Wett.

Bartica, B. G.; January 17, 1924; Linder 739.

Botanic garden, Paramaribo, Surinam; November 1, 1923; Linder 319.

*PERICHAENA CHRYSOSPERMA* Lister.

Plantation Vryheid, B. G.; February 28, 1924; Linder 995.

Obviously a list of this sort can not enumerate the myxomycetous flora of the region, for in a general collection such as that made by Dr. Linder, it is impossible to gather a large number of species of every order; and the Myxomycetes, because of their inconspicuousness and the difficulties incidental to their gathering and preservation in the field, are collected even less than the other groups. As a result, tropical specimens are poorly represented in most herbaria and each collection adds a little more to our knowledge of their geographic distribution. For that reason this list, even though small, is of some general interest.

## A NEW GENUS OF THE SUBFAMILY NITSCHKIEAE

R. CIFERRI

(WITH 1 TEXT FIGURE)

The specimen on which the present contribution is based is a part of the Reliquiae Massanae preserved in the Mycological Herbarium of the School of Viticulture and Enology in Alba (Italy), and lacks reference to host and locality, and time of collection.

The fungus was found on dry decorticated branches. The perithecia are coriaceous-carbonaceous, black, cupulate, not distinctly ostiolate, composed of a nearly hemispherical superior ascigerous cavity and of a sterile basal part, of plectenchymatous tissue. The perithecia and hyphae are furnished with black, sharp-pointed and minutely tuberculated prickles and have a very light to nearly brilliant black metallic iridescence. The ascus contains eight ellipsoidal or ovoidal, inordinate, brownish or blackish, smooth, continuous spores.

Following the complete monograph on the subfamily Nitschkieae by Fitzpatrick (1), the fungus in its possession of perithecial prickles is near to the genus *Acanthonitschkea* Spegazzini (2). It differs from it in spore characters and bears the same relation to *Tympanopsis* Starbäck (3) that *Acanthonitschkea* bears to *Nitschkia*. Since no genus in the group will include the species, it seems desirable to found for it a new genus and this is dedicated to the monographer of the subfamily, H. M. Fitzpatrick.

**Fitzpatrickia** n. gen. (clar. mycol. H. M. Fitzpatrick dic.)

Astromaticus; peritheciis superficialibus hemisphaericis, aggregatis, leviter tuberculatis, indistincte ostiolatis, coriaceo-carbonaceis, nigris, spinulatis; hyphis subculi iridescentenigrescentibus, ramificatis, septatis, leviter tuberculatis, parce spinulatis; ascis clavatis, aparaphysatis, octosporis; ascosporis irregulariter dispositis, brunneolis vel fuscis, continuis, levibus; spinis rigidulis, erectis vel suberectis, continuis, non ramificatis, acutis, nigro-iridescentibus.

*Fitzpatrickia Massae* n. sp. (memoria Doct. Caroli Massae dic.)

Peritheciis aggregatis, rotundato-cupulatis, nigris, iridescentibus, leviter tuberculatis, indistincte ostiolatis,  $320-460\ \mu$  diam., spinulatis; ascis clavatis vel elongato-clavatis,  $19.5-27 \times 5-8.5\ \mu$ , aparaphysatis, octosporis, mox evanescentibus; ascosporis ellipsoideis vel ovoideis, saepe inequilateralis, non seriatis, continuis,



FIG. 1. Ascus with spores    FIG. 2. Ascospores

flavo-brunneis usque ad fuscidulis,  $7.5-9 \times 2.5-3.5\ \mu$ , levibus; hyphis brunneis vel nigrescentibus, armatis; spinis elongatis, rigidulis, continuis, non ramificatis, leviter tuberculatis,  $140-190 \times 8-15\ \mu$ .

Hab. in ligno decorticato, leg. C. Massa in loco indeterminato.

The specimen is preserved in the Mycological Herbarium at Alba, Italy.

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## THE FRUITING OF COLLYBIA DRYOPHILA IN PURE CULTURE<sup>1</sup>

R. F. POOLE

(WITH PLATES 5 AND 6)

*Collybia dryophila* was obtained in pure culture by planting on prune agar, mycelium and rhizomorphs from dewberry roots, where it is parasitic. The mycelium grew abundantly on a variety of acidified culture media, including sweet potato, apple, rice, and barnyard manure, but mushrooms were not obtained on any of the above media. They developed slowly on cooked, dead, dewberry roots, but became very abundant after prune medium was added. The plants were very uniform in such characters as color, composition and arrangement of parts, and varied only in physical sizes.

The definite uniformity of the plants was so striking as to indicate that similar studies of related strains on a standard medium may lead to a definite understanding and explanation of the confusion and uncertain definition that exists in such groups as *Collybia dryophila* and *Armillaria mellea* at the present time. It is hoped that the results presented in this paper on the fruiting of *Collybia dryophila* in pure culture may be of some assistance to those interested in studying other related strains and groups in pure culture.

The culture medium used in growing the mushrooms was prepared by cutting, into small sections, parts of dead roots and canes of the dewberry. To this was added 200 cc. of prune medium, which was prepared by cooking 150 grams of large prunes in a liter of water. The combined prune dewberry medium in two-liter flasks was sterilized for one hour at the ordinary temperature and pressure. Cultures were started in July, August, and October. They were maintained at 25 degrees centigrade in candle light. There was simultaneous and abun-

<sup>1</sup> Published with the approval of the Director of the North Carolina Experiment Station as paper number 21 of the Journal Series.

dant growth of both mycelium and rhizomorphs. They became very prominent after fourteen days, when a large amount of spawn was transferred to the flasks.

The first production of plants was obtained in 30 days, but maximum production was obtained after a growth of 60 to 90 days. In a two-liter flask, 17 well-developed mushrooms were grown in two months. In six flasks 58 mushrooms were removed in the same period. The cultures have continued to produce new rhizomorphs and mushrooms for six months.

Although the mature caps varied from 2 to 8 cm. in diameter, the extremes were rare, and the average was between 4 and 5 cm. Sometimes when 8 to 12 plants were developed together in the same flask some were more vigorous than others (PLATE 5, FIG. 1). The color changes were always uniform. The same facts were noted in the form, size and arrangement of the gills, flesh and heterogenous tissues. The sizes of caps and stems were the notable differences.

TABLE I  
MEASUREMENTS OF ELEVEN PLANTS GROWN FROM DECEMBER 23 TO 27 IN A TWO-LITER FLASK

Plant	Pileus (diameter) cm.	Stipe (length) cm.	Stipe (diameter at base) mm.	Stipe (diameter at cap) mm.
1	8.0	10	20	12
2	4.5	11	10	7
3	4.5	10	15	6
4	6.0	10	12	11
5	4.5	10	12	7
6	4.5	10.5	11	7
7	4.0	9	12	8
8	3.2	9.5	7	5
9	3.0	9	9	5
10	2.5	7	9	4
11	3.0	7	8	2

The measurements of eleven plants grown in single flasks from December 23 to 27 are given in table I. In this yield of eleven plants the caps varied from 2.5 to 8 cm. in diameter but five of this number measured only from 4.0 to 4.5 cm. The eleven stems measured from 7 to 11 cm. in length, while seven varied from only 9 to 10 cm. The bulb-like base varied from 8 to 20 mm. in diameter. The even stem near the cap varied from 2 to 12 mm., but seven stems measured from 4 to 7 mm.

The rate of growth of the mushrooms was readily followed in the flasks, where the first signs were small fuzzy-like tumors on the rhizomorphs. These were mostly numerous, but only a few got any larger than the button, where they wilted and dried. The matured plants grew slowly in the beginning and for the first 48 hours. Later growth was quite rapid in comparison to the first two days. The stems developed thickness at first and during this period of development the tomentose covering was prominent on the young stems. This became less distinct as the stem extended in length, but the striations which were faint the first two days became very pronounced during the next 48 hours. The stem of the young plant was mostly white and began to tan and brown from the base upward as the plant matured. It sometimes became equal in color to the cap. The smaller size of the stem at maturity was due to the rapid development of the stem into length only a few hours previous to maturity.

TABLE II  
THE RATE OF DEVELOPMENT OF THREE *Collybia dryophila* MUSHROOMS IN  
PURE CULTURE

Plant no.	Date	Time	Pileus (diameter) mm.	Stipe (length) mm.	Stipe (diameter at base) mm.	Stipe (diameter at cap) mm.
1	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 A.M.	5	11	4	3
	" 14	5 P.M.	7	15	4	3
	" 15	10 A.M.	10	27	4	2
	" 16	10 A.M.	16	50	3	2
2	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 A.M.	9	20	5	4
	" 14	5 P.M.	10	22	6	5
	" 15	10 A.M.	28	57	6	4
	" 16	10 A.M.	44	90	6	3
5	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 P.M.	12	25	10	5
	" 14	5 P.M.	15	26	12	5
	" 15	10 A.M.	30	65	12	7
	" 16	10 A.M.	54	100	12	6

The growth of the cap can be described as gradual and uniform from the beginning. The form, somewhat gibbous, was main-

tained throughout. The cap was whitish for one day, then it became light tan and shades of brown in 24 hours, and afterwards changed only slightly to a deeper brown in the center. The greatest growth of the cap was during the last 48 hours before maturity which was always between 4 to 5 days.

The development of the mushroom is best observed in plate 5, figure 1, where the new tumor-like button and one- and two-day-old plants, photographed 10 A.M., December 24, are shown. Successive figures 2 and 3 show the later developments of the same plants at 10 A.M. on December 25 and 26, respectively.

Some measurements on different days of plants in another flask are given in table II. The most interesting part of these data is the rapid growth of the plants during the second 48 hours, between January 14 and 16. Although the complete development of the plant was attained in 4 to 5 days, best sporing occurred when the plants were not disturbed for 48 hours longer. The plants began to slowly dry out after they were fully matured.

Sometimes there was an unequal development of subcespitose plants on the same rhizomorph (PLATE 5, FIG. 1). The two plants on the extreme left were the same one and two described in table II. In the beginning these plants were of equal size throughout, but at maturity the stem of plant 1 was 40 mm. shorter and the cap 28 mm. less in diameter than plant 2. This is not surprising since there were 21 buttons in this flask January 12, and only 8 matured, counting plant 1, which was below normal size. Uniformity of growth was not always interfered with when two plants were developed side by side from the same rhizomorph as is shown in figure 2. The greatest uniformity of size, as well as other characters, was obtained when solitary plants were grown (Fig. 3).

#### DESCRIPTION OF PLATES

##### PLATE 5

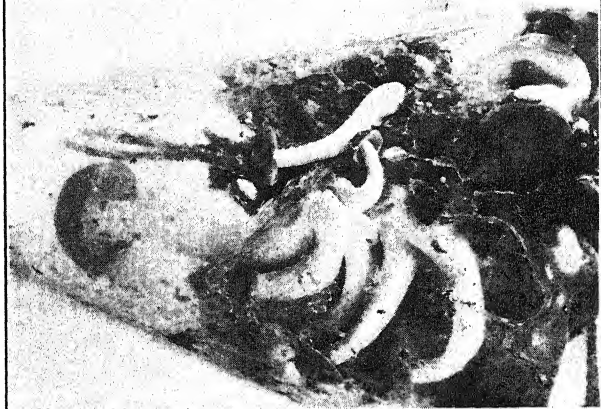
Fig. 1. Buttons and small mushroom growing on old dewberry roots saturated with prune medium in two-liter flasks. Photographed 10 A.M., December 24.

Fig. 2. The same mushrooms photographed 10 A.M., December 25.

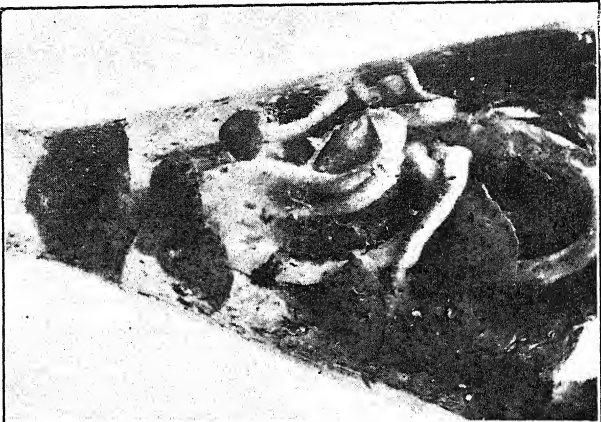
Fig. 3. The same mushrooms photographed 10 A.M., December 26.



1



2



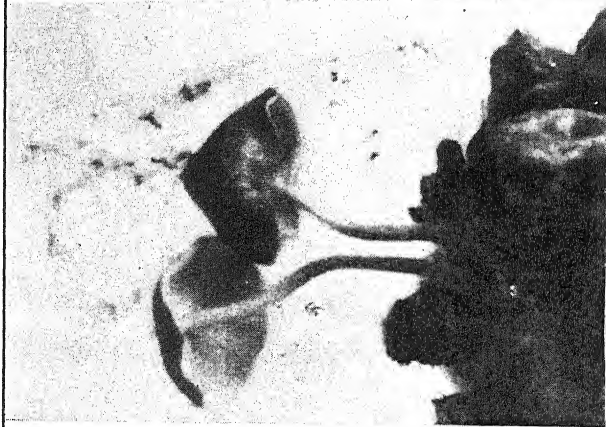
3

COLLYBIA DRYOPHILA





1



2



3

COLLYBIA DRYOPHILA





PLATE 6

Fig. 1. Mushrooms of different sizes on same rhizomorphs. Subcespitose plants on extreme left show big variation in size at maturity although of same size in the beginning.

Fig. 2. Mushrooms, however, may attain equal size without one interfering with the other in growth.

Fig. 3. A solitary mushroom.

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## THE UREDINIA OF CRONARTIUM COMANDRAE AND MELAMPSORA MEDUSAE

E. H. Moss

Among the rusts (Uredinales) the family Melampsoraceae is of great interest because of the variety of host relationships and the diversity of forms exhibited by its various members. Attempts have been made to give expression to natural affinities among genera of the family, but, unfortunately, many of these attempts have been based upon superficial observations or upon incorrect interpretations of certain morphological characters. It is apparent, therefore, that a more thorough investigation of these rusts should precede further endeavors to express natural relationships in a phylogenetic classification or otherwise.

In a recent paper (4), attention was directed to the uredinial stage of one subfamily of the Melampsoraceae, namely, the Pucciniastreae, and it was concluded (contrary to certain earlier reports) that the uredinia of representative forms of this subfamily are strikingly similar in their mode of development. The present communication deals with a member of each of two other subfamilies of the Melampsoraceae, namely, the Cronartieae and the Melampsoreae, and the main conclusion reached is that the uredinia of these species develop in essentially the same manner as in the Pucciniastreae. In connection with the following descriptions of *Cronartium Comandrae* and *Melampsora Medusae*, it has been considered unnecessary to provide illustrations, because very similar stages in development are fully figured in an earlier paper (4).

### CRONARTIUM COMANDRAE PECK

This species was obtained on *Comandra Richardsiana* Fernald in the jack-pine forest of central Alberta. Uredinia were collected on July 22, 1926, fixed in medium chromacetic acid, and subsequently embedded in paraffin. Microtome sections were stained with safranin and light green, a combination that

proved very satisfactory for the interpretation of young uredinia.

The uredinium develops from a hyphal plexus beneath a stoma of the host leaf. Vertical hyphae arise in palisade fashion under the epidermis and become divided each into three enlarged cells, namely, peridial, intercalary and sporogenous cells. It is probable that peridial and intercalary cells are sister cells, but convincing evidence of this has not been found in my preparations. At this early stage, the uredinium closely resembles that of *Uredinopsis*, as figured in an earlier paper (4). The peridial cells have small nuclei and large vacuoles; the intercalary cells have degenerate nuclei and cytoplasm that stains faintly; and the sporogenous cells are characterized by large nuclei and deeply staining cytoplasm. At a slightly later stage in development, the intercalary cells collapse, due to the outward growth of the basal sporogenous cells. Thus the intercalary cells function as space-formers and also as "disjunctive" cells (Kursanov's term), serving to separate the peridial from the sporogenous cells. A conjugate nuclear division then takes place, one pair of daughter nuclei passing into the "bud," after which a septum is formed between the "bud" and the mother cell. The bulbous cell which thus arises from the basal cell is a spore-initial; following further expansion and conjugate nuclear division, it becomes divided by a transverse wall into stalk cell and young spore. Before the spore thus formed has reached maturity, a second spore-initial may arise by a "budding" process from the basal cell, and still later additional spores may develop from the same sporogenous cell. As a consequence of the pressure exerted by the developing spores, the peridial cells become somewhat flattened and the epidermis bulges outwards, and finally the epidermal and peridial cells that cover the central part of the sorus are ruptured, thus allowing the spores to emerge.

It should be pointed out that the foregoing description differs from that given by Colley (1) for *Cronartium ribicola* Fischer in one important respect. Colley does not recognize in the uredinium a layer of disjunctive cells, although his illustration of a young sorus shows three-celled columns with subterminal cells that closely resemble the disjunctive cells as I have observed them in *Cronartium Comandrae*. The subterminal cells are

interpreted by Colley as the first spore-initials, and no mention is made of disjunctive cells in connection with the separation of the peridium from the underlying spore-initials and spores.

#### MELAMPSORA MEDUSAE THÜM.

A collection of young uredinia of this species was made in the Timagami Forest Reserve, Ontario, on August 29, 1925. Some of the material was carefully fixed in the field and later used in making preparations suitable for the study of the sori.

The early stages in development of the uredinium are essentially the same as described above for *Cronartium*. The peridial and intercalary cells of the three-celled columns are considerably smaller and less conspicuous than in *Cronartium* and have a very transitory existence. These cells are, however, quite as easily observable in the young sorus as are the corresponding cells in the uredinium of *Hyalopsora* (4). Spore-initials arise from the basal cells by the characteristic "budding" process, and the pressure exerted by the growth of the first spore-initials effects the early collapse of the intercalary cells. Unlike *Cronartium Comandrae* and the Pucciniastreae, *Melampsora Medusae* produces paraphyses in its uredinium. These organs seem to arise, in the first instance, from the border of the rather young uredinium after a number of spores have been formed in the central part. The paraphyses arch over the developing spores, forming a fringe round the mouth of the newly opened sorus. During the development of the peripheral paraphyses the peridial cells become more or less crushed, and for this reason these cells can best be observed in the very young uredinium. Later, paraphyses commonly develop in the central part of the sorus as well as towards the border. The paraphyses seem to arise from basal cells, either peripheral ones which are not sporogenous, or more central ones which have already produced one or more spore-initials. Whether the paraphysis is separated from the basal cell by a septum is difficult to ascertain owing to the constricted nature of the lower end of the paraphysis, but such a septum does not appear to exist.

The uredinium of *Melampsora* has been generally considered to be without a peridium. However, as long ago as 1899,

Klebahn (2) observed that certain species of *Melampsora*, namely, *M. populina* and *M. Lini*, have evanescent peridia. Klebahn was inclined to think that a peridium is absent in the uredinium of *M. Laricis-capraearum* of the willow. More recently Kursanov (3) has described a uredinial peridium for *Melampsora Helioscopiae* Winter on *Euphorbia* and has concluded, from an examination of mature uredinia of *Melampsora* on *Salix* and *Populus*, that peridia are also present in these species. There is abundant evidence therefore that a peridium characterizes the uredinium of *Melampsora*, but whether disjunctive cells occur below the peridium is a matter requiring further investigation. According to my observations, disjunctive cells are formed in *M. Medusae*, but according to Kursanov no such cells separate the peridium from the sporogenous cells in *M. Helioscopiae*.

#### DISCUSSION

Representatives of the Pucciniastreae (4), Cronartieae and Melampsoreae that have been examined by the writer are essentially alike in the mode of development of their uredinia, having in common the following features:

- (1) A peridium is present and consists of the terminal cells of three-celled columns which arise in the young sorus.
- (2) The basal cells of the columns give rise to spore-initials by upward growth or "budding."
- (3) The intercalary cells of the columns disorganize and serve to separate the peridium from the basal cells and to make space for the initial growth of the first spore-initials.

It is true that marked differences exist among the uredinia of these rusts, for example, differences in size and shape of the mature pustule, in spore characters, in degree of persistence and differentiation of the peridium. These characters are undoubtedly of the greatest diagnostic value in taxonomy, but from a phylogenetic point of view they are probably of secondary importance in comparison with the fundamental features that have been emphasized in this paper. Even the paraphyses of *Melampsora*, particularly if these are to be regarded as the homologues of spore-initials or of spores, may be of less importance in connection with natural relationships than is the evanescent peridium with its associated disjunctive cells.

The bearing of the conclusions of this paper upon the question of affinities among the rusts, and upon the problem of the relative primitiveness of various types of sori will be determined only as a result of further investigation in the Melampsoraceae, including the subfamily Chrysomyxaeae, as well as in other groups of the Uredinales.

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## NOTES ON ARCTIC UREDINALES<sup>1</sup>

J. C. ARTHUR

The plants brought back from northwestern Greenland in the Danish Jubilee Expedition of 1920-23 have been critically searched for micromycetes by Mr. J. Lind, resulting in the detection of 80 species, 5 of them being Uredinales. Mr. Lind has made his report in No. 2 of the account of the expedition (1926) and takes occasion to revise some of the taxonomy involved. As to the rusts it is only required to state here that the writer agrees entirely with the conclusions, except in the case of *Puccinia Thlaspeos*, which he believes to be quite distinct from *P. Holboellii*. The characters of the two species are given in volume 7, page 532, of the North American Flora, a publication, by the way, that is not mentioned in the literature cited.

In a later work, "The Geographical Distribution of Some Arctic Micromycetes," 1927, Mr. Lind has made a highly important contribution to the subject of plant dissemination in high northern latitudes. He points out that the strong wind of the region may carry seeds, fragments of stems and leaves for long distances over the surface of snow and ice, often depositing them in crevices or valleys where conditions are favorable for growth upon return of warm weather. For nine months of the year ice bridges the waters between islands and even the continents, so that the winds may sweep parts of flowering plants with the endophytes living upon them over a smooth dry surface in a circumpolar migration, even over such long stretches as between Greenland, Norway, Nova Zembla and Spitzbergen. This deduction is supported by citations from the writings of a number of arctic travelers. In all probability the agency of the wind is the chief, if not the only, agency for the dispersal of plants and their parasites in arctic regions, a conclusion which seems logical.

It is also pointed out that the most common arctic rusts are short-cycle species, like *Puccinia Arenariae*, *P. Holboellii*, *P.*

<sup>1</sup>Contribution from the Department of Botany, Purdue University Agricultural Experiment Station.

*Drabae* (*P. Cochleariae*), *P. Cruciferarum* (*P. Cardamines-bellidifoliae*), which are readily distributed in connection with their hosts. Nine such species are known from near the arctic circle in North America.

The author's statement regarding heteroecious species, however, requires emendation. He says, "If heteroecious species of rusts were found in arctic regions, or other species of fungi the nature of which demanded that spores from one host-plant should be transferred to another in order to thrive, it would be a proof against my theory that the endophytes are spread together with their hosts in arctic countries." This statement carries the inference that heteroecious rusts require two unlike hosts in order to thrive, which is only true for a comparatively few species, for many of them can be spread and maintained indefinitely by their urediniospores.

To account for the presence of *Melampsora arctica*, which occurs on both *Salix* and *Saxifraga*, and is one of the most abundant of arctic rusts, the author assumes that portions of infected *Saxifraga*, the mycelium being perennial, will carry the rust, and upon taking root again can spread fresh spores to the willows in the vicinity. It is equally conceivable, however, that the uredinial mycelium in the willow stems may survive the winter and spread the rust directly to other willows. That method of propagation is known to be common with *Melampsora Bigelowii* in the Rocky Mountains and elsewhere.

But neither of these methods will account for the abundance in arctic regions of the heteroecious *Puccinia Polygoni-vivipari*, which has no perennial mycelium in either alternate host. The aecia which occur on *Ligusticum* have not been found as far north as the uredinia and telia. The latter were taken on Cockburn Island, far north of the arctic circle. Probably the rust is scattered by its urediniospores, which are abundant and could easily be distributed by the wind along with the seeds and dried stems of the host.

Another rust of a still different character is the autoecious *Uromyces carnea* (*Aecidium Phacae-frigidae*) which has been collected in the aecial form near the mouth of the Mackenzie River, far north of the arctic circle. The aecia arise from a



perennial mycelium, the telia are only slightly pulverulent and are not accompanied by uredinia. In this case the distribution is doubtless by means of wind, carrying the teliospores along with fragments of the plant until lodged against other plants which can be infected when new growth begins. It is improbable that the aecia play the part in distribution that they do in the willow rust, as the branches of *Phaca* can scarcely retain their vitality and start new growth as can readily be done with *Saxifraga*.

There are nine species of long-cycle rusts known from near the arctic circle in North America, the same number as of short-cycle rusts, but with the exception of *Melampsora arctica* they are not so abundant or on so many species of hosts. All of the nine species are heteroecious except two, *Uromyces carnea* and *Trachyspora Alchemillae*. In reviewing the possibilities for distribution of these long-cycle forms there seems to be no doubt that they conform in general to the same method as the short-cycle forms and are dependent upon the wind for long distance migrations.

PURDUE UNIVERSITY,  
LAFAYETTE, INDIANA

## AN INTERESTING FERN RUST NEW TO THE UNITED STATES

H. W. THURSTON, JR.

On May 21, 1927, several members of the Department of Botany of the Pennsylvania State College, while botanizing on a rocky hillside near Howard, Pennsylvania, found a wonderfully fine stand of the common polypody, *Polypodium vulgare* L. Literally hundreds of these ferns, in fact almost every one examined, had been attacked by a rust, which was fruiting in great abundance on the over-wintered fronds and in many instances producing very noticeable necrosis.

A rust on *Polypodium vulgare* has been described by Bell,<sup>1</sup> from Ontario, Canada, who noted its occurrence on "fronds of the previous summer," and named it *Uredinopsis polypodophila*. Bell also suggested the possible connection of this rust with a *Peridermium* on the 2-8-year-old leaves of *Abies balsamea* which he found in close association with the fern rust and to which he gave the name *Peridermium pycnograndis*. Faull and Watson<sup>2</sup> transferred the fern rust to the genus *Milesia*. Arthur<sup>3</sup> has accepted the connection proposed by Bell and has proposed the combination *Milesia pycnograndis* (Bell) Arth.

The station was revisited and collections made on May 30, June 24, and July 14, 1927. The first collection yielded only urediniospores which agreed well with Bell's description. In the later collections a few telia were found scattered within the epidermal cells. Measurements of these were made from fixed and stained preparations and the telia were found to be  $14-21 \times 36-55 \mu$  which differs slightly from the measurements reported by Arthur in the North American Flora, namely,  $16-27 \times 24-45 \mu$ .

A search of the hillside and vicinity failed to reveal the presence of *Abies*, which has never been known to occur in this region.

<sup>1</sup> Bot. Gaz. 77: 25. 1924.

<sup>2</sup> Manuscript 1925.

<sup>3</sup> N. Am. Flora 7: 685. 1925.

The question, therefore, arises in the absence of specific cultures whether *Abies* is actually the alternate host, and if so, how this rust perpetuates itself in the absence of the alternate host. On July 14, 1927, infections were observed on new fronds, of the current season. Since these fronds live over the winter, it is conceivable that late summer infections by the parasite live over winter in the form of dormant mycelium, or that the urediniospores themselves live over and do not germinate until the following spring. A somewhat similar case is found in the behavior of *Hyalopsora Polypodii* (DC.) Magn. on *Filix Fragilis*.

PENNSYLVANIA STATE COLLEGE,  
STATE COLLEGE, PENNA.

## NOTES AND BRIEF ARTICLES

Dr. C. L. Shear of the Bureau of Plant Industry, Washington, D. C., is spending a good part of the winter making a mycological and pathological survey of the Hawaiian Islands and expects to bring back a large collection of fungi for study.

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Dr. Frederick A. Wolf, Pathologist, Fruit Disease Investigations, United States Department of Agriculture, for the past two years stationed at Orlando, Florida, has accepted a position at Duke University, Durham, North Carolina.

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Dr. Jaroslaw Peklo, Professor of Applied Botany and Phytopathology at the Check Technical University, Prague, has come to the United States in order to pursue his studies in mycology, phytopathology and breeding of plants resistant to disease. He will be in this country about nine months and is at present at the Bureau of Plant Industry, Washington, D. C.

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"Die Pilze Mitteleuropas," edited by H. Kniep, P. Claussen, and J. Basz, published by Dr. Werner Klinkhardt, Leipzig, 1927. Price 5 Marks per Lieferung.

Three folio parts of this illustrated work on the fleshy fungi of Central Europe have already appeared. The fungi are fully described and illustrated in colors from photographs taken from fresh specimens. The illustrations are of unusual excellence and are not confined to single specimens but include a number of individuals of different ages and conditions. Detailed drawings of basidia, cystidia and spores are also given. The three parts which have already appeared are devoted to species of *Boletus*. It is intended to publish the parts at intervals of two or three months. This work ought to be of great usefulness to all mycologists, mushroom collectors and others interested in fungi.

C. L. SHEAR.

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In the second paper on "Additions to the Australian Ascomycetes," Dr. Ethel McLennan and Isabel Cookson, of the

University of Melbourne, have reported three species of *Lamprospora* which were collected and named by Dr. Fred J. Seaver from material collected in and about The New York Botanical Garden. The three species are as follows: *Lamprospora tuberculata* Seaver; *Lamprospora tuberculatella* Seaver; *Lamprospora Maireana* Seaver. An Australian variety of a fourth species has been established, *Lamprospora areolata* Seaver, var. *australis* McLennan and Cookson. Two of the above named species are known only in New York and Australia. Misses McLennan and Cookson indicate their intention of continuing their survey of the Discomycetes of Australia and it is very interesting to note the cosmopolitan habits of some of these fungi.

#### FIFTH INTERNATIONAL BOTANICAL CONGRESS, CAMBRIDGE, 1930

At the International Congress of Plant Sciences (Fourth International Botanical Congress) held at Ithaca, United States, in August, 1926, an invitation was conveyed from British Botanists for the Fifth International Botanical Congress to be held in England in 1930. The invitation was accepted by the Botanists assembled at Ithaca, and arrangements are now being made for the Congress to be held at Cambridge about the middle of August, 1930.

An Executive Committee has been formed to make arrangements for the Congress, consisting of Dr. F. F. Blackman, Prof. V. H. Blackman, Dr. E. J. Butler, Prof. Sir John Farmer, Prof. F. E. Fritsch, Prof. Dame Helen Gwynne-Vaughan, Dr. A. W. Hill, Prof. W. Neilson Jones, Sir David Prain, Dr. A. B. Rendle (Treasurer), Prof. A. C. Seward (Chairman), Prof. W. Stiles, and Prof. A. G. Tansley.

It has been decided to organize the Congress in the following seven sections: Morphology (including Anatomy), Palaeobotany, Plant Geography and Ecology, Taxonomy and Nomenclature, Genetics and Cytology, Physiology, and Mycology and Plant Pathology.

Mr. F. T. Brooks, The Botany School, University of Cambridge, England, and Dr. T. F. Chipp, Royal Botanic Gardens, Kew, England, have been appointed Honorary Secretaries of the Congress, and any communications with regard to the Congress should be addressed to one or other of the Secretaries.



LARS ROMELL

# MYCOLOGIA

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No. 2

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## LARS ROMELL

C. L. SHEAR

(WITH PLATE 7)

It was with the deepest regret that I learned from Mr. L. G. Romell of the death of his father which occurred on August 13, 1927. I take pleasure in complying with Mr. Romell's request that I prepare a biographical sketch of his father for MYCOLOGIA, of which he was an Associate Editor. My acquaintance with Doctor Romell began with correspondence and exchange of specimens thirty-five years ago and I had the pleasure of visiting him three times in Stockholm and making several collecting trips with him about Stockholm and Upsala.

Doctor Romell was born at Kumla province of Närke, Sweden, December 4, 1854. I unfortunately have little information in regard to his early life. His deep interest in the fungi apparently developed during his university training. After the usual preliminary training he entered the University of Upsala and received the degree of Bachelor of Arts in 1885. In 1886 he was appointed professor at the College of Norra Latinläroverket, at Stockholm, which position he occupied until 1887, when he became adjunct master at Östermalms Läroverk in Stockholm. In 1890 he took up work as a patent attorney and this was his occupation until his death.

His deep interest in the fungi was aroused and stimulated by H. von Post of Upsala who had been associated with the great mycologist Elias Fries, and had made a large collection of colored plates of fungi which had been determined by Fries himself. In [MYCOLOGIA for January-February (20: 1-47) was issued January 3, 1928]

this way Romell became imbued with the spirit and traditions of Fries. He therefore, on account of his access to the illustrations and personal knowledge supplied him by von Post, and the unpublished material of Fries, was unusually equipped for making critical interpretations of the Friesian species of the higher fungi.

Doctor Romell never neglected an opportunity to do mycological work. Whether his devotion to the fungi ever led him to neglect his work as a patent attorney we can not say. In any case his field excursions undoubtedly improved his physical condition and increased his capacity for office work. During my visits in Sweden he always insisted on accompanying me on collecting trips. This was a great pleasure and benefit as he was familiar with all the good collecting localities and his enthusiasm and knowledge of the higher fungi were very enjoyable and helpful. His intimate acquaintance with the higher fungi was derived largely from his field work. He was an acute observer and recognized the distinctive characters between closely related species as they occur in the field at different times and under different conditions. He spent much time during his later years in helping to arrange the collections of fungi in the Swedish State Museum of Natural History which contains a great collection of Doctor Rehm and also the Bresadola Herbarium. The latter he purchased with his own funds and deposited in the museum herbarium. He also accumulated a large private herbarium of fungi by collection and exchange, and had a large number of photographs of fungi, and several thousand microscopical preparations. A list of his publications, which includes thirty-five or more titles, can not be included here for lack of space. Besides his unpublished papers he issued two centuries of fungi exsiccati, chiefly from Scandinavia. Most of his critical studies were devoted to the Hymenomycetes, especially *Polyporus* and *Russula*.

His alma mater, the University of Upsala, conferred the Doctor's degree *honoris causa* upon him in 1927, but the promotion was not to take place until September. His laurel crown from the faculty was presented, however, at his funeral.

His son to whom we are indebted for most of the information in this sketch tells me that he was very much interested in spiritualism and occultism. His early plans when leaving home



were to become a pastor or missionary. During his University career, however, he abandoned his fundamentalist ideas and previous plans. On account of his unorthodox teaching he was obliged later to give up that profession. During the world war he bought and distributed large numbers of pamphlets opposing the conflict. He also tried to bring influence to bear in opposition to the war by writing to the Empress of Germany and to the Pope. In connection with these activities his son remarks: "He had, alas, too great a belief in the power of reason, truth, and right in the world."

By Doctor Romell's death, mycology has lost an enthusiastic and able worker, and the world an ardent advocate of truth and justice.

BUREAU OF PLANT INDUSTRY,  
WASHINGTON, D. C.

## STUDIES IN TROPICAL ASCOMYCETES—IV SOME HYPOCREALES FROM TRINIDAD

FRED J. SEAVER

(WITH PLATES 8-12)

During the winter and spring of 1921 the writer obtained a rather large collection of fungi from the island of Trinidad of which only the rusts have been reported up to this time.<sup>1</sup>

The fungi of other groups have been determined so far as possible but no attempt has been made to publish them in full. Since a number of interesting Hypocreales have come to hand it is thought worth while to report on some of these now and they will be mentioned in order of their interest.

Probably to me the most interesting was the marble-like growth on the stems of some grass or possibly young bamboo. This has the appearance of an insect gall but differs in that it is very lightly attached to its host, and really constitutes a globose stroma of a Hypocreaceous fungus, rusty-red or yellowish in color, the color varying with age. Cushion-like areas represent the fertile portion, a large part of the surface remaining sterile.

This was at first referred by us to the genus *Hypocrella* which its fruiting character would suggest. Later it was identified by Mr. T. Petch of Ceylon as *Ascopolyporus polychrous* Möller. The name *Ascopolyporus* was apparently suggested because of the resemblance of the stroma to the sporophore of a *Polyporus* with its upper sterile and lower fertile surface. In our specimens the fertile areas do not seem to be confined to the lower surface. Except for the smaller size our collection compares very favorably with the colored illustration by Möller.<sup>2</sup>

A single collection of this species was made in Trinidad, consisting of one mature stroma about a centimeter in diameter, one

<sup>1</sup> Arthur, J. C. Uredinales collected by Fred J. Seaver in Trinidad. *Mycologia* 14: 12-24. 1922.

<sup>2</sup> Möller, A. Phycomyceten und Ascomyceten; Untersuchungen aus Brasilien 300. pl. 3, f. 41-44. 1901.

about half this size and two smaller ones. From the ease with which they become detached we assume that this genus like its close relative *Hypocrella* is not parasitic on the plant host but probably entomogenous, although there was no direct evidence of insect hosts. The dried plants are decidedly hard and woody, another character which may have suggested the name *Ascopolyporus*.

So far as we are aware this is the first time the species has been collected in Trinidad, previous records having been confined to Brazil, the type locality. The species was originally reported on bamboo. Our specimen was on some undetermined grass, possibly young bamboo.

The next most interesting species was *Hypocrella viridans* (Berk. & Curt.) Petch. This species was described by Berkeley and Curtis from material collected in Cuba and was based on sterile specimens. For this reason it was placed with the doubtful species of *Hypocrea* by the writer in North American Flora (3: 35. 1910). Petch<sup>3</sup> was the first to observe and record the perfect stage of this fungus, placing it in its correct genus. He gives for the species the following synonyms: *Hypocrea viridans* Berk. & Curt.; *Aschersonia viridans* (Berk. & Curt.) Pat.; *Aschersonia disciformis* Pat.; *Hypocrea amazonica* Cooke; and *Aschersonia viridula* Sacc. All of these names refer to the *Aschersonia* stage. Material collected by Dr. Roland Thaxter in Trinidad apparently furnished data for the connection between the *Aschersonia* and the *Hypocrella* stages.

Our own material which was later determined by T. Petch of Ceylon was collected in abundance on the leaves of an aroid, *Anthurium aripoense*, in the vicinity of the guacharo caves. The stromata are thickly scattered over the leaves of the plant host and decidedly green. The perithecia which are present in many of the stromata are numerous and apparently subsuperficial. Whether they become so with age or are normally developed on the surface of the stroma could not be determined but this character is likely to be misleading since the perithecia in the plants of this genus are usually immersed.

<sup>3</sup> Petch, T. Studies in Entomogenous Fungi: II. The Genera *Hypocrella* and *Aschersonia*. Ann. Royal Bot. Gard. Perad. 7: 236. 1921.

The establishment of the connection between the imperfect and perfect stages of this fungus adds to our knowledge of the flora of North America as recorded in North American Flora, in that it removes one species, *Hypocrea viridans*, from the doubtful list, enabling us to place it in its proper genus.

A third species of especial interest is *Hypocrella Andropogonis* (P. Henn.) Petch. While originally described from material collected in Brazil on *Andropogon*, our specimens were collected in Trinidad on a sedge, *Rynchospora cephalotes* (L.) Vahl, although the host had not been determined when the fungus was named by T. Petch. The two plant hosts are so far removed from each other that we suspect the insect hosts may also be very different and that a more critical study may reveal morphological differences in the fungi as well. For the present, however, our Trinidad specimens of *Hypocrella* on *Rynchospora* are referred *Hypocrella Andropogonis* which has previously been known only on *Andropogon*. Petch cites as synonyms of this species *Aschersonia Andropogonis* P. Henn. and *Aschersonia parasitica* P. Henn.

Our material was found to be quite abundant on the leaves of the above named sedge, as many as ten or twelve stromata occurring on a single leaf, the largest ranging up to 5 mm. in diameter. Often several are fused together. They are of a whitish color and while most of them show pycnidia only, several stromata were found which showed the ascigerous stage well developed. Mr. T. Petch in determining the species spoke of it as being rather immature. This species had been previously collected in Trinidad by Dr. Thaxter. It is now known from Brazil, Paraguay and Trinidad.

Another species which was collected in abundance in Trinidad is *Macbridella striispora* (Ellis & Ev.) Seaver, which was formerly known only from the type collection in Nicaragua and one collection from Porto Rico. The abundant collection from Trinidad has given us the opportunity to make a more extended study of this species.

In May, 1926, Mr. E. W. Mason, Assistant Mycologist of the Imperial Bureau of Mycology of Kew, England, sent the writer some of the ascigerous stage of *Sphaerostilbe Musarum* Ashby which, at Mr. Ashby's suggestion, had been compared with the

type of *Sphaerostilbe longiascus* Möller and found to be the same, except for some minor discrepancies in color, etc.

At Mr. Mason's request this was compared with the type of *Macbridella striispora* (Ellis & Ev.) Seaver and also found to be the same. When the genus *Macbridella* was established for this species the stilbaceous character of the fungus had been overlooked. In fact the conidial stage seemed to be entirely wanting in the specimens examined although present in abundance in the later collections from Porto Rico and Trinidad. This, however, would not affect the validity of the genus provided the brown, striate characters of the spores are sufficient grounds for its segregation from the old genus *Sphaerostilbe*. Also Ellis's specific name has priority over the other names mentioned above. The synonymy of this species would then be as follows:

MACBRIDELLA STRIISPORA (Ellis & Ev.) Seaver, Mycologia 1: 196.  
1909.

*Nectria striispora* Ellis & Ev.; C. L. Smith, Bull. Lab. Nat. Hist.  
Univ. Iowa 2: 398. 1893.

*Sphaerostilbe longiascus* Möller, Phyc. Ascom. Unters. Brasilien  
122. 1901.

*Sphaerostilbe Musarum* Ashby, Bull. Dept. Agr. Jamaica, N. S.  
2: 112. 1913.

The species is characterized by the very large cylindrical perithecia which are attenuated at the apex. The perithecia are partially covered with a yellow coating of very short and poorly developed tomentum which is more or less evanescent. The upper part of the perithecium is naked and red, the shade varying as in other species of nectriaceous fungi with age and conditions. The ascospores are very large, pale brown at maturity and marked with the peculiar striations which are characteristic of many of the tropical ascomycetes. The conidial stage, or the remains of it, is very conspicuous in some of the material although the perithecial stage is often collected without it. According to Mr. Ashby the conidial stage is easily obtained from the ascospores in culture.

The specimen of *Sphaerostilbe Musarum* which we examined was reported on *Cacao*. Our specimens from Trinidad were

collected on the bark of some undetermined tree. It so closely resembles the former that I suspect it was also on *Cacao*.

*Scolecnectria tetraspora* was described by the writer in North American Flora based on a specimen collected by F. S. Earle on *Cacao* trunks in Jamaica, the species at that time being known to me only from the type collection. A second collection of this fungus was made by the writer in Trinidad on partially matured pods of *Cacao*. The species is characterized by the groups of perithecia which are associated with a *Verticillium* which may be its conidial stage although no attempt has been made to prove the connection because of the age of the material. The individual perithecia are light yellow and characterized by the coarse bran-like particles with which they are covered. As indicated by the specific name, the asci are 4-spored, another diagnostic character.

Occurring as this fungus does on the blighted fruits of the *Cacao*, I suspect that it may be partially responsible for their blighting and may prove to be of economic importance. In the herbarium of The New York Botanical Garden, I find a specimen which is identical with my species listed by Massee from Grenada under the name of *Calonectria flavida*. I have been unable to locate the place of publication of this species and suspect that it may never have been published.<sup>4</sup> Even though it has, the name is untenable since we have in North America another species by the same name.

Another interesting species listed in North American Flora, from Martinique and Jamaica, is *Nectria rhytidospora* Pat. As the name implies, this species is characterized by the striated spores. In connection with our work on the fungi of Porto Rico, numerous specimens of this species were encountered. The perithecia, however, showed so much variation in appearance and color that they were identified with difficulty. In some cases they are smooth and red with a conspicuous ostiolum while in others they are covered with sulphur-yellow powder and with the ostiolum rather inconspicuous. Our Trinidad collection showed both types of perithecia on the same substratum and this together with the fact that the ascospores from the different types

<sup>4</sup> Miss E. M. Wakefield of Kew Gardens has, in a recent letter, confirmed this suspicion.

of perithecia were identical has led the writer to assume that the apparent difference is due merely to variation. The yellow covering which is occasionally present consists of club-shaped hairs which appear to be rather evanescent. For the present at least we are regarding the different types of perithecia as representing merely phases of the same species, although later investigations might prove otherwise.

*Nectria suffulta*, originally known from Cuba and later from various islands of the West Indies and Mexico, was collected in Trinidad, the latter specimens occurring on rotting wood and conforming well with the other specimens examined, except that the spores are somewhat smaller. This difference, however, is scarcely sufficient in our opinion to distinguish it specifically. The species is characterized by the large collapsing perithecia and the conspicuous fasciculated hairs and striated ascospores. *Nectria setosa* Ferd. & Winge is a synonym.

A fine collection of *Stilbocrea intermedia* (Ferd. & Winge) Seaver, a species formerly known from Louisiana and the West Indies, was found in Trinidad on the bark of some tree. The species is characterized by its *Hypocrea*-like stroma and its *Stilbum*-like conidial stage.

#### NEW SPECIES

##### *Podocrella* gen. nov.

Like *Podocrea* but with filiform spores.

##### *Podocrella poronioides* sp. nov.

Stromata stipitate, the stem gradually expanding above into a fruiting head the fertile upper surface of which is nearly plane or slightly convex, the whole structure somewhat resembling a *Poronia*, brownish-black in color; perithecia strongly protruding, giving the upper surface a papillate appearance; asci reaching a length of 250  $\mu$ , 8-spored; spores filiform, reaching a length of 65–75  $\mu$  and a diameter of 2.5  $\mu$  in the center, gradually attenuated toward either end, about 15-septate (the septa rather difficult to count).

On rotten wood among mosses in the vicinity of Valencia, March 4, 1921 (*Seaver 3017*).

*Nectria indusiata* sp. nov.

Perithecia isolated but thickly scattered over the upper surface of the leaf, minute, bright red and very rough with bran-like granules, erumpent through the epidermis which is pushed up and usually persists in the form of an indusium or lid; asci clavate, 8-spored, reaching a length of 120–150  $\mu$  and a diameter of 20  $\mu$ ; spores fusoid, slightly s-shaped, 3-septate, hyaline, 55–80  $\times$  7–8  $\mu$ .

On a fallen leaf of *Micropolis* sp., Morne Bleu, March 13, 1921 (*Seaver* 3176).

In the preparation of this paper the writer is indebted to Mr. T. Petch of Ceylon; Dr. Roland Thaxter of Harvard University and Miss E. M. Wakefield of Kew Gardens, England, for aid in identifying material; also to Dr. N. L. Britton and Mr. Percy Wilson of our own institution for determination of hosts.

THE NEW YORK BOTANICAL GARDEN,  
BRONX, NEW YORK CITY

## EXPLANATION OF PLATES

## PLATE 8

Figs. 1–3. *Podocrella poronioides*: 1–2, habitat sketches enlarged; 3, asci and spore removed from the ascus.

Figs. 4–5. *Ascopolyporus polychrous*: 4, habitat sketch a little enlarged; 5, portion of ascus with spores.

Figs. 6–8. *Macbridella striispora*: 6, habitat sketch enlarged; 7, ascus with spores; 8, conidiospore.

## PLATE 9

Figs. 1–3. *Hypocrella viridans*: 1, habitat sketch about natural size; 2, pycnidial stroma with pycnidia arranged in a circle; 3, pycnosporos removed from pycnidia.

Figs. 4–8. *Hypocrella Andropogonis*: 4, habitat sketch about natural size; 5, pycnidial stroma enlarged; 6, pycnosporos removed; 7, ascigerous stroma enlarged; 8, portion of ascus with spores.

## PLATE 10

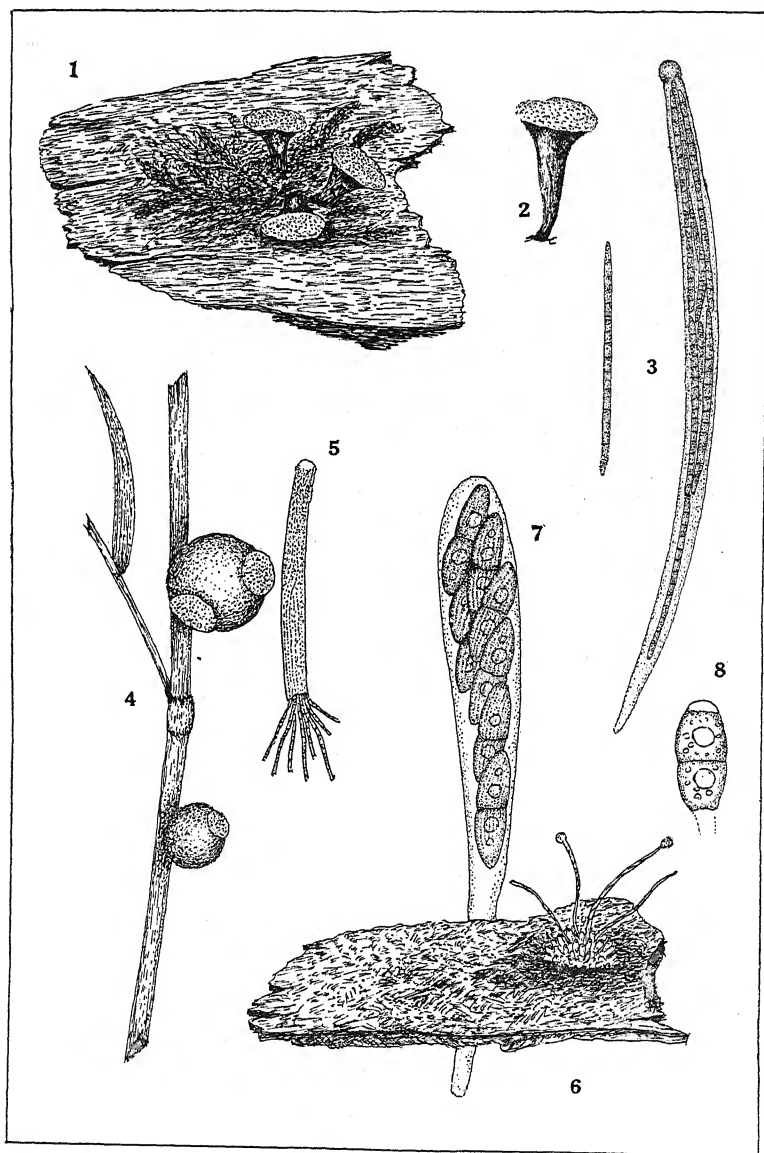
Figs. 1–3. *Calonectria indusiata*: 1, habitat sketch about natural size; 2, perithecia enlarged; 3, ascus with spores.

Figs. 4–6. *Hypocrella viridans*: 4, habitat sketch about natural size; 5, ascigerous stroma enlarged; 6, ascus with one spore removed.

## PLATE 11

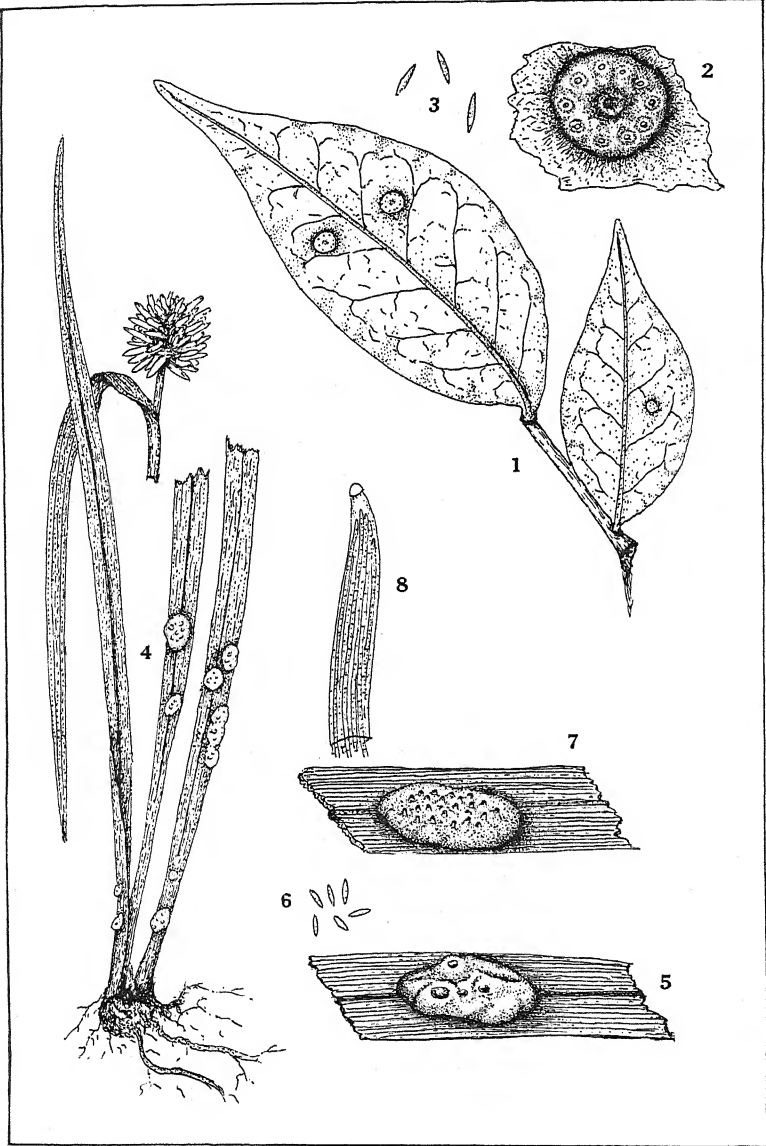
Figs. 1–4. *Scoleconectria tetraspora*: 1, habitat sketch about natural size; 2, cluster of perithecia enlarged; 3, individual perithecia isolated; 4, ascus with spores.





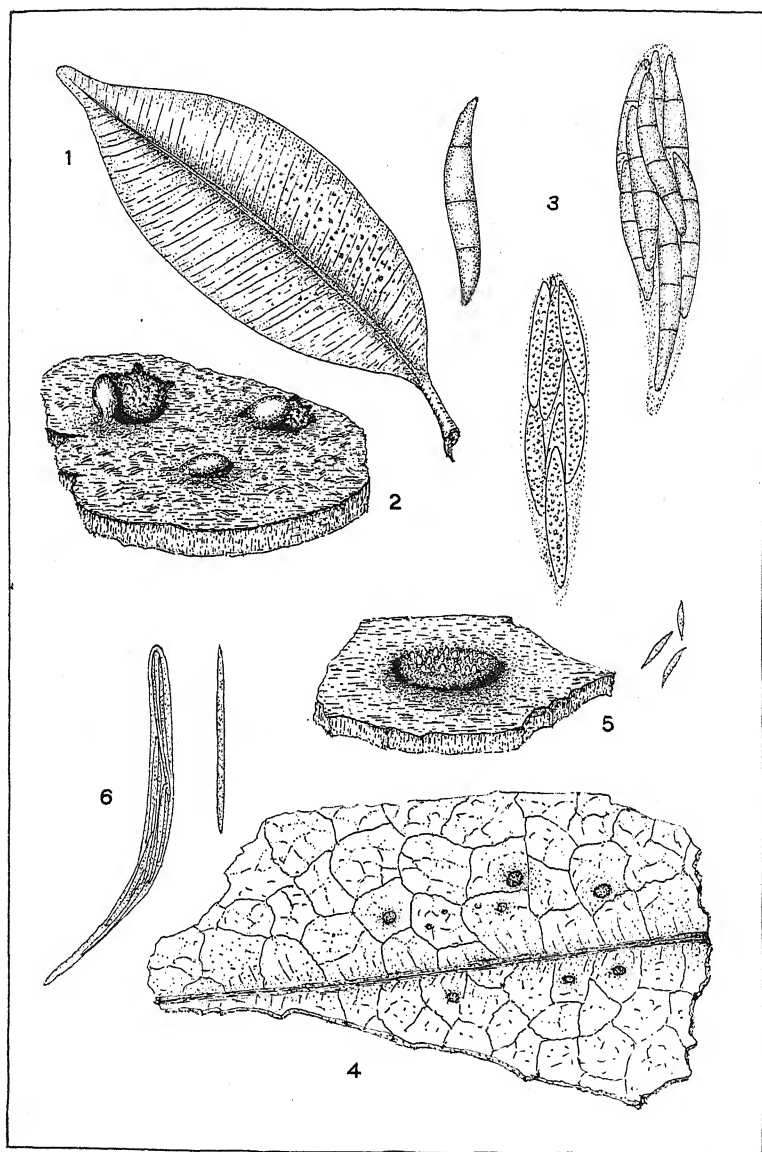
1-3. *PODOCRELLA FORONIOIDES*  
 4-5. *ASCOPOLYPORUS POLYCHROUS*  
 6-8. *MACBRIDELLA STRIISPORA*





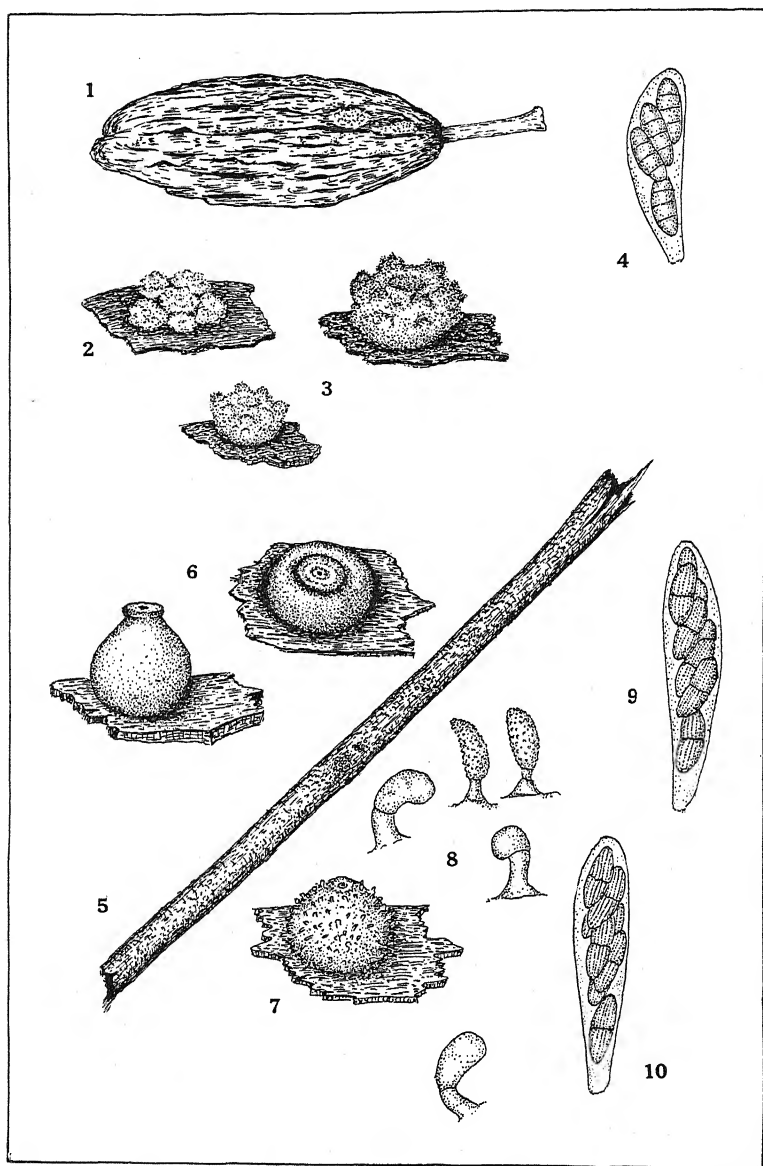
1-3. *HYPOCRELLA VIRIDANS*  
4-8. *HYPOCRELLA ANDROPOGONIS*



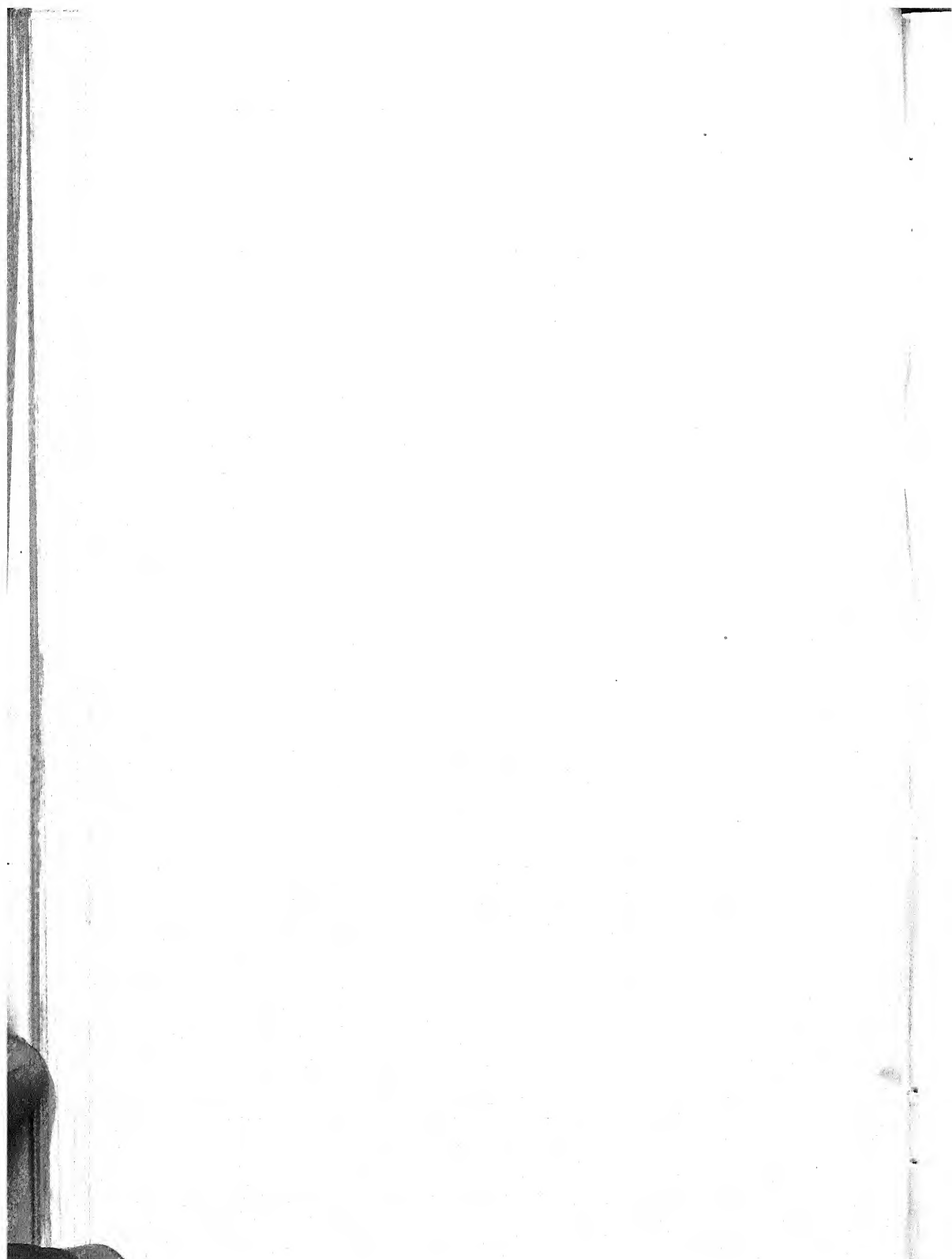


1-3. *CALONECTRIA INDUSIATA*  
4-6. *HYPOCRELLA VIRIDANS*

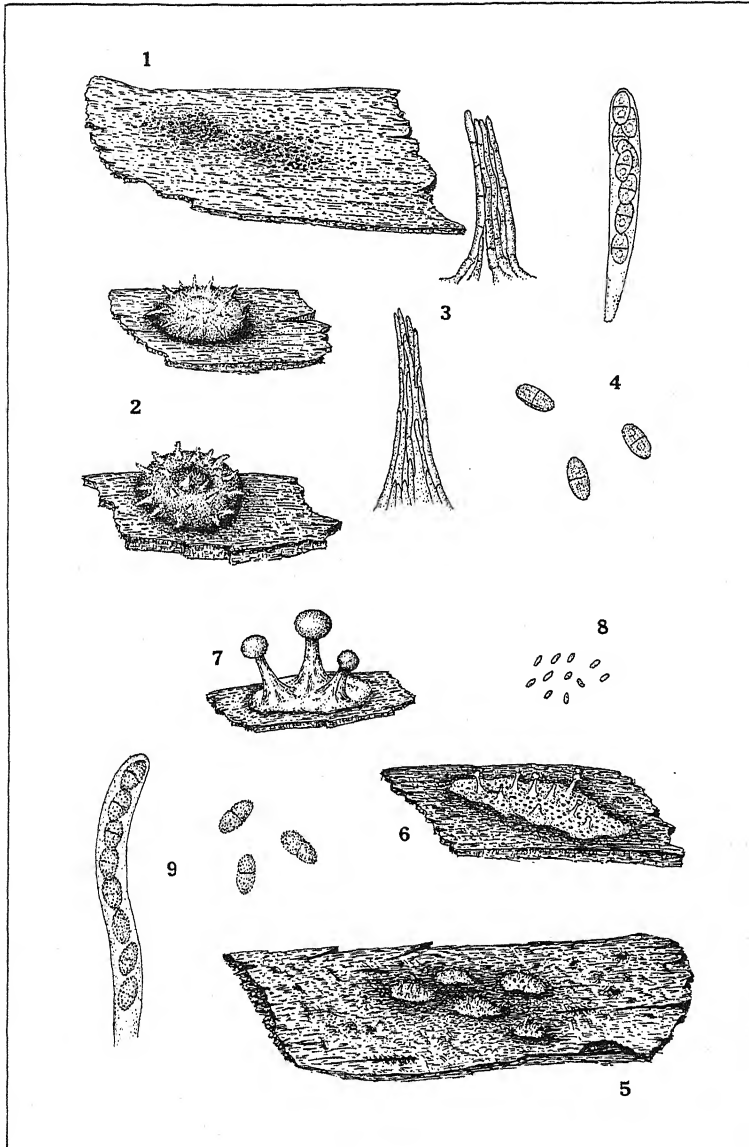




1-4. *SCOLECONNECTRIA TETRASPORA*  
5-10. *NECTRIA RHYTIDOSPORA*







1-4. *NECTRIA SUFFULTA*

5-8. *STILBOCREA INTERMEDIA*



Figs. 5-10. *Nectria rhytidospora*: 5, habitat sketch about natural size; 6, perithecia isolated, smooth type; 7, perithecium with yellow hair-like structures; 8, hair-like structures from perithecium; 9-10, asci with spores.

## PLATE 12

Figs. 1-4. *Nectria suffulta*: 1, habitat sketch about natural size; 2, perithecia isolated and enlarged; 3, fasciculate hairs from perithecium; 4, ascus and ascospores.

Figs. 5-9. *Stilbocrea intermedia*: 5, habitat sketch about natural size; 6, stroma enlarged; 7, conidial stage enlarged; 8, conidia; 9, ascus and ascospores.

Microscopic drawings made with the aid of the camera lucida.

## FUNGI OF SANTO DOMINGO—II. UREDINALES<sup>1</sup>

FRANK D. KERN

A botanical expedition was made to Santo Domingo in the spring of 1926 by Rafael A. Toro, of the Insular Experiment Station, Porto Rico, and the writer. We reached the island March 9 and left April 1. The trip was made possible by the co-operation of the Department of Agriculture and Labor of Porto Rico and the University of Porto Rico. All kinds of fungi were collected. The writer was especially interested in the rusts. Altogether about 400 numbered collections were made.

In a paper entitled "Fungi of Santo Domingo—I" (*Mycologia* 19:66-84, 1927) Toro has reported on the collections of Phycomycetes, Ascomycetes, and Fungi Imperfecti. His paper reports 97 species. Prior to this paper, published reports of the fungi of Santo Domingo have been scattered and are comparatively meager. In his paper, Toro reviews very thoroughly the literature dealing with Santo Domingan fungi.

The present paper reports the collections of rusts made by Kern and Toro. In the following list our names and the year are omitted for our collections. In addition to our collections, I am including some made by Doctor M. F. Barrus, of Cornell University, who collected in Santo Domingo during January and February, 1926. Since the publication of this paper has been delayed, I am able to include some specimens collected in May, 1927, by Mr. Carlos E. Chardon, Commissioner of Agriculture of Porto Rico. Doctor Barrus and Mr. Chardon have generously turned over to me their specimens for study and record. Full data are included for specimens by other collectors. A few specimens are included which were contributed by Doctor R. Ciferri, Director of the Agricultural Experiment Station of Santo

<sup>1</sup> Read before the Mycological Section of the Botanical Society of America at the Philadelphia meeting, December 29, 1926. Contribution from the Department of Botany, The Pennsylvania State College, No. 60. Published by permission of the Director of the Agricultural Experiment Station, No. 444.

Domingo. We are further indebted to Doctor Ciferri for assistance and many courtesies extended while we were in Santo Domingo. We are likewise indebted to Mr. Rafael A. Espaillet, Secretary of Agriculture of Santo Domingo, and to Mr. Santiago Michelena, of Santo Domingo. For aid in the determination of hosts, I am indebted to Doctor N. L. Britton and Mr. Percy Wilson, of The New York Botanical Garden, Doctor P. C. Standley, of the United States National Museum, Professor A. S. Hitchcock, and Mrs. Agnes Chase, of the United States Department of Agriculture. To all of these and to the administrative officers of the Department of Agriculture and Labor of Porto Rico and of the University of Porto Rico, thanks are due and are hereby most heartily accorded.

Previous reports of rusts from Santo Domingo are to be found chiefly in the North American Flora, Volume 7, and in a series of papers written jointly by Doctor R. Ciferri and Doctor Romualdo Gonzalez Fragoso and published in the Boletin de la Real Sociedad Española de Historia Natural. These papers are entitled "Hongos Parasitos y Saprofitos de la Republica Dominicana." Eleven papers have appeared up to the present (June, 1927). Thirty rusts have been reported in these two series, 18 in the North American Flora and 12 in the Fragoso-Ciferri papers. Of these 30 species which have been previously reported, 17 are in the list collected by us and 13 are to be found in a supplementary list headed "Species Previously Reported from Santo Domingo, not in the Foregoing List." When a different name is used from the one in the previous report, explanations are included.

During the study of these Santo Domingan collections, the papers dealing with the rust floras of Cuba<sup>2</sup> (Arthur & Johnston) and Porto Rico<sup>3</sup> (Whetzel & Kern) have been most helpful. Consulting these lists has suggested a comparison of the distribution of rusts in these three neighboring islands. The total number of rusts here reported for Santo Domingo, 86, seems small compared with 140 for Cuba and 181 for Porto Rico. This is doubtless due to the fact that the explorations in Santo Domingo have been much less thorough than in the other islands. It has

<sup>2</sup> Memoirs Torrey Club 17: 97-175. 1918.

<sup>3</sup> Sci. Sur. Porto Rico and Virgin Islands 8: 111-144. 1926.

been very interesting to me to find that of the 86 species known from Santo Domingo, 56 are known in Cuba and 70 in Porto Rico while 11 are not known in either Cuba or Porto Rico. The following table presents the situation in tabular form by genera.

Genus	Total No. of Species in S. D.	No. of These Species in Cuba	No. of These Species in P. R.	No. not in Cuba or P. R.
<i>Coleosporium</i> ..	2	2	2	
<i>Phakopsora</i> .....	3		2	1
<i>Crossopora</i> .....	1	1	1	
<i>Cerotium</i> .....	2	1	2	
<i>Endophyllum</i> ...	2	1	2	
<i>Puccinosira</i> .....	1	1	1	
<i>Botryorhiza</i> .....	1	1	1	
<i>Ravenelia</i> .....	6	3	4	1
<i>Prospodium</i> ....	2	1	1	1
<i>Tranzschelia</i> ....	1	1	1	
<i>Kuehneola</i> .....	1	1	1	
<i>Desmella</i> .....	1	1	1	
<i>Uromyces</i> .....	11	8	9	2
<i>Puccinia</i> .....	39	29	34	3
<i>Aecidium</i> .....	2	1	1	
<i>Uredo</i> .....	11	5	7	3
Totals .....	86	56	70	11

For the sake of brevity no attempt has been made in this list to include synonyms. In order that anyone interested may find the synonymy, descriptive accounts, and distribution, I have included in parentheses under nearly every species a reference to the North American Flora. It has seemed wise to give this citation since at present there is not available an index to Volume 7 of the North American Flora. If a different genus is used in the Flora that information is given and if there is additional data in the "Additions and Corrections" a second reference appears. It is hoped that this will aid materially in tracing the status of the various species in this list. If no reference to the Flora is given, that species is not there reported.

1. COLEOSPORIUM ELEPHANTOPODIS (Schw.) Thüm. Myc. Univ. 953. 1878. (N. Am. Fl. 7: 89, 1907; 654, 1924.)

On *Elephantopus mollis* H.B.K., San Cristobal, March 14, II, 91.

2. *Phakopsora dominicana* sp. nov.

On *Croton angustatus* Urban, San Jose de Las Matas, May 8, 1927, C. E. Chardon 397.

III. Telia hypophyllous, gregarious, on hypertrophied spots 1–1.5 mm. in diameter, crowded, sometimes confluent, irregularly orbicular, 0.2–0.3 mm. across, pulvinate, blackish; teliospores united in a compact mass, appearing obscurely catenulate, with 2–7 or more cells in a series, each spore elliptical or cuboidal,  $13\text{--}17 \times 16\text{--}30 \mu$ , the wall smooth, smoky,  $1\text{--}1.5 \mu$  thick, apical wall of outer spores  $3\text{--}4 \mu$ .

This species differs from *Phakopsora Crotonis* and *P. mexicana* in having smaller spores with thinner and different colored walls. The sori are also much smaller. It is a striking fact that no urediniospores are to be found on this specimen. On the upper side of some of the spots there is some indication of pycnia, but none could be identified in sections.

3. *Prospodium Tabebuiae* sp. nov.

On *Tabebuia* sp., Santiago, March 22, 1926, II, Kern & Toro 30.

II. Uredinia hypophyllous, scattered, minute, roundish, 0.1 mm. or less across, early naked, chocolate-brown, ruptured cuticle not evident; paraphyses numerous, united at the bases, fusiform,  $10\text{--}14 \times 32\text{--}40 \mu$ , slightly incurved, the wall about  $1.5 \mu$  thick, hyaline, smooth; urediniospores globoid,  $20\text{--}24 \times 24\text{--}28 \mu$ , the wall indistinctly laminate, the inner portion chestnut-brown, about  $2 \mu$  thick, overlaid with thickly irregular hyaline papillae reaching out  $2\text{--}2.5 \mu$ , the pores distinct, 2, opposite and equatorial.

III. Telia not seen.

Although no telia have been found, the character of the uredinial structures together with the host relationship indicates that this is a species of *Prospodium*. It differs from *P. appendiculatum* and *P. Amphiphilii*, which are also on Bignoniaceae, in having the paraphyses united at the bases. It differs from *P. plagiopus*, also on Bignoniaceae, in not having a thick gelatinous layer in the urediniospore-wall. It differs from *P. bahamense* in the thickness and markings of the urediniospore-wall.

4. *CROSSOPSORA NOTATA* Arth. N. Am. Fl. 7: 695. 1925.

On *Byrsonima crassifolia* H.B.K., Santo Domingo, March 25, II, 86.

5. CEROTELIUM DESMIUM (Berk. & Br.) Arth. N. Am. Fl. 7: 698. 1925.

On *Gossypium* sp., San Pedro de Macoris, March 10, II, 41.

*Gossypium barbadense* L., San Francisco de Macoris, August, 1925, R. Ciferri 858 (specimen—reported in Bulletin Estac. Agron. Haina (R. D.), p. 3, 1926).

Reported by Fragoso & Ciferri (Bol. R. Soc. Esp. Hist. Nat. 25: 443, 1925) as *Kuehneola Gossypii* (Lagerh.) Arth. on *Gossypium barbadense*.

6. CEROTELIUM FICI (Cast.) Arth. Bull. Torrey Club 44: 509. 1917. (N. Am. Fl. 7: 696, 1925.)

On *Ficus Carica* L., Haina, March 15, II, 89; Bonao, June 14, 1925, R. Ciferri 397.

Reported by Fragoso & Ciferri (Bol. R. Soc. Esp. Hist. Nat. 25: 357, 1925) as *Kuehneola Fici* (Cast.) Butler.

7. ENDOPHYLLUM CIRCUMSCRIPTUM (Schw.) Whetzel & Olive, Am. Jour. Bot. 4: 49. 1917. (N. Am. Fl. 7: 700, 1925.)

On *Cissus sicyoides* L., San Pedro de Macoris, March 10, 111; San Cristóbal, March 13, 96, March 12, 93; Bajabonico, March 23, 77; San Francisco de Macoris, Aug. 5, 1918, John A. Stevenson 7048.

The Stevenson collection is reported in the N. Am. Fl.

8. ENDOPHYLLUM STACHYTARPHETAE (P. Henn.) Whetzel & Olive, Am. Jour. Bot. 4: 50. 1917. (N. Am. Fl. 7: 701, 1925.)

On *Valerianodes cayennense* (L. C. Rich.) Kuntze, Puerto Plata, March 24, 82; Isabel de Torre, April 28, 1887, Eggers Phan. no. 1751.

The Eggers collection is reported in the N. Am. Fl.

9. PUCCINIOSIRA PALLIDULA (Speg.) Lagerh. Tromsø Mus. Aarsh. 16: 122. 1894. (N. Am. Fl. 7: 127, 1907; 702, 1925.)

On *Triumfetta semitriloba* Jacq., San Cristóbal, March 12, O, III, 114; Puerto Plata, March 24, 83; San Pedro de Macoris, March 10, 110, Jan. 23, 1926, M. F. Barrus 3. *Triumfetta* sp., La Vega, May 4, 1927, C. E. Chardon 349.



10. BOTRYORHIZA HIPPOCRATEAE Whetzel & Olive, Am Jour. Bot. 4: 47. 1917. (N. Am. Fl. 7: 703, 1925.)  
On *Hippocratea volubilis* L., Santo Domingo, March 27, 1926, 126.
11. RAVENELIA CAESALPINIAE Arth. Bull. Torrey Club 31: 5. 1904. (N. Am. Fl. 7: 141, 1907; 714, 1925.)  
On *Mimosa Ceratonia* L., river north of Bonao, May 4, 1927, C. E. Chardon 345.
12. RAVENELIA INDIGOFERAE Tranz. Hedwigia 33: 369. 1894. (N. Am. Fl. 7: 144, 1907.)  
On *Indigofera mucronata* Spreng., La Vega, March 19, II, 12; Bajabonico, March 23, II, III, 11.  
*Indigofera suffruticosa* Mill., Santiago, March 20, II, 20; San Cristobal, March 14, II, 21.
13. RAVENELIA INGAE (P. Henn.) Arth. N. Am. Fl. 7: 132. 1907.  
*Ravenelia Whetzelii* Arth. Mycologia 9: 64. 1917. (N. Am. Fl. 7: 707, 1925.)  
On *Inga Inga* (L.) Britton, San Cristobal, March 14, O, I<sup>u</sup>, II, 92, II (on seedlings), 90.
14. RAVENELIA LONCHOCARPI Lagerh. & Diet. Hedwigia 33: 46. 1894. (N. Am. Fl. 7: 717, 1925.)  
On *Lonchocarpus domingensis* DC., Bonao, March 16, II, 32.
15. RAVENELIA PORTORICENSIS Arth. Bull. Torrey Club 31: 5. 1904. (N. Am. Fl. 7: 139, 1907; 716, 1925.)  
On *Isandrina emarginata* (L.) Britton & Rose (*Cassia emarginata* L.), Barahona, Jan. 26, 1926, II, M. F. Barrus 8.  
This rust was originally described from a specimen collected by A. A. Heller in 1902 at Ponce, Porto Rico. It has been reported previously from Santo Domingo, having been found on a phanerogamic specimen in The New York Botanical Garden, Barahona, May, 1910, II, Pater Fuertes 192. It is known also from Cuba, Haiti, and Jamaica (see Mem. Torrey Club 17: 118, 1918).

16. *RAVENELIA SPINULOSA* Diet. & Holw. Bot. Gaz. 31: 336.  
1901. (N. Am. Fl. 7: 140, 1907; 716, 1925.)  
On *Cassia crista* Jacq., Azua, March 29, II, 16.  
This rust has not been reported previously from the West Indies. It has been known from Mexico and Guatemala.
17. *PROSPODIUM APPENDICULATUM* (Wint.) Arth. Jour. Myc. 13:  
31. 1907. (N. Am. Fl. 7: 160, 1907; 725, 1925.)  
On *Tecoma Stans* (L.) H.B.K. (*Stenolobium Stans* Seem.),  
Santiago, March 20, 1926, II, 119.
18. *KUEHNEOLA MALVICOLA* (Speg.) Arth. N. Am. Fl. 7: 187.  
1912. (N. Am. Fl. 7: 730, 1925.)  
On *Pavonia coccinea* Cav., Bani, March 28, II, 7.
19. *DESMELLA SUPERFICIALIS* (Speg.) Syd. Ann. Myc. 16: 242.  
1918. (N. Am. Fl. 7: 704, 1925.)  
On *Dryopteris subtetragona* (Link) Maxon, San Cristobal, II,  
March 13, 50.
20. *UROMYCES APPENDICULATUS* (Pers.) Fries, Summa Veg.  
Scand. 514. 1849. (*Nigredo*, N. Am. Fl. 7: 257, 1912;  
764, 1926.)  
On *Vigna repens* (L.) Kuntze, Puerto Plata, March 24, 56.
21. *UROMYCES COLUMBIANUS* Mayor, Mem. Soc. Neuch. Sci.  
Nat. 5: 467. 1913. (*Nigredo*, N. Am. Fl. 7: 771, 1926.)  
On *Melanthera* sp., Santo Domingo, March 27, II, 127.
22. *UROMYCES DOLICHOLO* Arth. Bull. Torrey Club 33: 27. 1906.  
(*Nigredo*, N. Am. Fl. 7: 258, 1912; 765, 1926.)  
On *Cajan Cajan* (L.) Millsp., San Cristobal, March 12, II, 112;  
Azua, March 15, 1913, Rose, Fitch & Russell Phan. no.  
3920.  
The Rose, Fitch, Russell collection is reported in the N. Am. Fl.
23. *UROMYCES GEMMATUS* Berk. & Curt.; Berkeley, Jour.  
Linn. Soc. 10: 357. 1869. (*Klebahnia*, N. Am. Fl. 7:  
479, 1921.)  
On *Jacquemontia nodiflora* (Desv.) G. Don, Santiago, March  
22, II, 25.

24. UROMYCES HEDYSARI-PANICULATI (Schw.) Farl.; Ellis, N. Am. Fungi 246. 1879. (*Nigredo*, N. Am. Fl. 7: 248; 761, 1926.)  
On *Meibomia scorpiurus* (Sw.) Kuntze, La Vega, March 17, II, 15; San Pedro de Macoris, Jan. 30, 1926, II, *M. F. Barrus* 10.  
*Meibomia cana* (Gmel.) Blake, Santo Domingo, March 27, II, 27.
25. UROMYCES IGNOBILIS (Syd.) Arth. Mycologia 7: 181. 1915. (*Nigredo*, N. Am. Fl. 7: 746, 1926.)  
On *Sporobolus indicus* (L.) R. Br., Puerto Plata, March 24, II, 57; San Cristobal, March 12, 39; Haina, March 11, 46; road Moca to Salcedo, May 7, 1927, *C. E. Chardon* 360.
26. UROMYCES LEPTODERMUS Syd.; Syd. & Butler, Ann. Myc. 4: 430. 1906. (*Nigredo*, N. Am. Fl. 7: 224; 746, 1926.)  
On *Lasiacis divaricata* (L.) Hitchc., Santiago, March 22, II, 28; San Cristobal, March 14, II, III, 23.  
*Panicum barbinode* Trin., San Cristobal, March 12, II, 37, 51; Haina, March 11, II, 44, 45; Bajabonico, March 23, II, 66; San Jose de las Matas, May 8, 1927, II, *C. E. Chardon* 398; San Pedro de Macoris, Feb. 1, 1926, II, *M. F. Barrus*.  
Reported by Fragoso and Ciferri on *Panicum maximum* as *Uredo Panici-maximi* Rangel, Haina, September, 1925 (Bol. R. Soc. Esp. Hist. Nat. 25: 509, 1925). This name is regarded as a synonym of *Uromyces leptodermus* by Arthur (Proc. Amer. Phil. Soc. 44: 206, 1925).
27. UROMYCES PIAUHYENSIS P. Henn. Hedwigia 47: 266. 1908. (*Uromyces*, N. Am. Fl. 7: 589, 1922.) March 20, II, 129.  
On *Wedelia reticulata* DC., Santiago, March 22, II, 131; La Vega-Moca road, March 20, II, 130; Puerto Plata, March 24, II, 128.  
*Wedelia Ehrenbergii* Schlch., road to Salcedo, May 7, 1927, *C. E. Chardon* 357.

28. UROMYCES PROËMINENS (DC.) Pass. Rab. Fungi Eur. 1795.  
1874. (*Nigredo*, N. Am. Fl. 7: 259, 1912; 765, 1926.)

On *Chamaesyce hirta* (L.) Millsp., Haina, March 30, II, 71;  
Puerto Plata, March 24, II, 61; San Cristobal, March  
14, II, 24, March 11, 9; San Pedro de Macoris, Feb. 1,  
1926, II, *M. F. Barrus* 21; road Moca to Santiago, May  
4, 1927, II, *C. E. Chardon* 355.

*Chamaesyce hypericifolia* (L.) Millsp., Haina, March 30, 70;  
Puerto Plata, March 24, I, II, 62; San Cristobal, March  
14, I, II, 52; road Moca to Salcedo, May 7, 1927, I, II,  
*C. E. Chardon* 359.

*Chamaesyce prostrata* (Ait.) Small, Samana, Aug. 2, 1918,  
*John A. Stevenson* 7015.

The Stevenson specimen is reported in the N. Am. Fl.

29. PUCCINIA ARACHIDIS Speg. Anal. Soc. Ci. Argent. 17: 90.  
1884. (*Bullaria*, N. Am. Fl. 7: 484, 1922.)

On *Arachis hypogaea* L., Haina, September, 1925, II, *R. Ciferri*.

30. PUCCINIA ARECHAVELATAE Speg. Anal. Soc. Ci. Argent. 12:  
67. 1881. (*Micropuccinia*, N. Am. Fl. 7: 541, 1922.)

On *Cardiospermum microcarpum* H.B.K., San Cristobal, March  
12, 38.

31. PUCCINIA CACABATA Arth. & Holw. Proc. Am. Phil. Soc. 64:  
179. 1925.

On *Chloris ciliata* Sw., Barahona, Jan. 27, *M. F. Barrus*;  
Bajabonico, March 23, 78.

*Chloris paraguayensis* Steud., Haina, March 30, 103.

This is apparently the first report of this rust from North  
America. The type locality is Bolivia, South America. These  
specimens have urediniospores with brown walls and equatorial  
pores like this species, not yellowish walls and scattered pores  
as in *Puccinia Chloridis* Speg.

32. PUCCINIA CANALICULATA (Schw.) Lagerh. Tromsö Mus.  
Aarsh. 17: 51. 1894. (*Dicaeoma*, N. Am. Fl. 7: 344, 1920;  
783, 1926.)

On *Cyperus ferax* L. C. Rich., San Cristobal, March 14, II, 17.

33. PUCCINIA CANNAE (Wint.) P. Henn. Hedwigia 41: 105.  
1902. (*Dicaeoma*, N. Am. Fl. 7: 380, 1920; 789, 1926.)  
On *Canna* sp., Haina, March 11, 134; Parque Independencia,  
Santo Domingo, March 9, 133; San Cristobal, March 13,  
132; Bonao, Aug. 15, 1918, *John A. Stevenson* 7004.  
The Stevenson specimen is reported in the N. Am. Fl.
34. PUCCINIA CENCHRI Diet. & Holw.; Holw. Bot. Gaz. 24: 28.  
1897. (*Dicaeoma*, N. Am. Fl. 7: 294, 1920; 775, 1926.)  
On *Cenchrus echinatus* L., Bajabonico, March 23, II, 69; Haina,  
March 11, 42; Barahona, Jan. 27, 1926, *M. F. Barrus*.  
*Cenchrus viridis* Spreng., Bajabonico, March 23, II, 64.
35. PUCCINIA CHAETOCHLOAE Arth. Bull. Torrey Club 34: 585.  
1907. *Uredo Chaetochloae* Arth. Bull. Torrey Club 33:  
518. 1906. (*Dicaeoma*, N. Am. Fl. 7: 288, 1920.)  
On *Paspalum Lindenianum* Steud., Santiago, March 22, II, 31.
36. PUCCINIA CYNODONTIS Lacroix, in Desmaz. Pl. Crypt. II.  
655. 1859. (*Dicaeoma*, N. Am. Fl. 7: 315, 1920.)  
On *Capriola dactylon* (L.) Kuntze, Santiago, March 20, II, 120;  
San Pedro de Macoris, Feb. 1, 1926, II, *M. F. Barrus*;  
Los Ranchos, May 7, 1927, *C. E. Chardon* 380.
37. PUCCINIA DICHROMENAE (Arth.) Jackson. *Uredo Dichro-*  
*menae* Arth. Bull. Torrey Club 33: 31. 1906. (*Dicaeoma*,  
N. Am. Fl. 7: 351, 1920.)  
On *Dichromena ciliata* Vahl, Bonao, March 16, 18.  
Telia have been found on *Dichromena colorata* in Bermuda and  
reported by Whetzel and Jackson. The telia are amphigenous,  
long covered by the epidermis. Arthur previously had referred  
the species to *Dicaeoma* (N. Am. Fl. 7: 351, 1920) but without  
any evidence. This proves that his surmise was correct.
38. PUCCINIA FUSCELLA Arth. & Johnston, Mem. Torrey Club 17:  
157. 1918. (*Bullaria*, N. Am. Fl. 7: 497, 1922.)  
On *Vernonia Spregeliana* Sch.-Bip., Santiago, March 22, 1926,  
II, III, 125.  
Apparently a new host. This rust heretofore known from

Cuba only. The teliospores are broad with a semihyaline thickening above.

39. PUCCINIA GOUANIAE Holw. Ann. Myc. 3: 21. 1905. (*Bullaria*, N. Am. Fl. 7: 487, 1922.)

On *Gouania polygama* (Jacq.) Urban, Bajabonico, March 23, II, 101; San Cristobal, March 13, II, iii, 97.

*Gouania lupuloides* (L.) Urban, San Francisco de Macoris, Aug. 5, 1917, John A. Stevenson 7027.

The Stevenson specimen is reported in the N. Am. Fl.

40. PUCCINIA HELICONIAE (Diet.) Arth. Bull. Torrey Club 45: 144. 1918. (*Puccinia*, N. Am. Fl. 7: 591, 1922.)

On *Bihai Bihai* (L.) Griggs, Santo Domingo, March 25, II, 88.

40. PUCCINIA HETEROSPORA Berk. & Curt. Jour. Linn. Soc. 10: 356. 1868. (*Micropuccinia*, N. Am. Fl. 7: 544, 1922.)

On *Bastardia viscosa* (L.) H.B.K., Santiago, March 21, 1926, 135.

*Gaya occidentalis* (L.) Sweet, Puerto Plata, June 4, 1906, C. Raunkiaer 1202a.

*Sida spinosa* L., Bajabonico, March 23, 67; Puerto Plata, March 24, 59; San Cristobal, March 13, 49.

*Sida urens* L., San Cristobal, March 12, 35.

*Sida* sp., Barahona, Jan. 26, 1926, M. F. Barrus 6.

The Raunkiaer collection is reported in the N. Am. Fl.

42. PUCCINIA HIBISCIATA (Schw.) Kellerm. Jour. Myc. 9: 110. 1903. *Caeoma* (*Aecidium*) *hibisciatum* Schw. Trans. Am. Phil. Soc. II. 4: 293. 1832. (*Dicaeoma*, N. Am. Fl. 7: 308, 1920; 728, 1926.)

On *Sporobolus Berteronianus* (Trin.) H. & C., Santiago, March 11, II, 29; Haina, March 11, II, 43.

This is the first report of this rust on the host here listed and its first report from the West Indies. It is common in the United States and extends into southern Mexico. Arthur reports *Uromyces Sporoboli* on this host from South America but the spores of this specimen are too small for that species. The thickness of the walls and the pore arrangement do not agree with *Uromyces ignobilis*.

43. PUCCINIA HYPTIDIS (Curt.) Tracy & Earle, Bull. Miss. Agric. Exp. Sta. 34: 86. 1895. (*Dicaeoma*, N. Am. Fl. 7: 408. 1921.)

On *Hyptis capitata* Jacq. (*Mesosphaerum capitatum* Kuntze), Bonao, March 16, 1926, II, 136; La Vega, March 19, II, 123.

44. PUCCINIA IMPEDITA Mains & Holw.; Arth. Mycologia 10: 135. 1918. (*Bullaria*, N. Am. Fl. 7: 493, 1922.)

On *Salvia occidentalis* Sw., San Cristobal, March 14, II, 54; March 13, II, 48; Haina, March 11, II, 47; San Pedro de Macoris, Jan. 20, 1926, II, *M. F. Barrus* 11.

45. PUCCINIA INFLATA Arth. Bull. Torrey Club 33: 516. 1906. (*Bullaria*, N. Am. Fl. 7: 486. 1922.)

On *Stigmaphyllon lingulatum* (Poir.) Small, Puerto Plata, March 24, II, 137; Santiago, March 20, II, 118; La Romana, April 1, II, 102; San Pedro de Macoris, Jan. 22, 1926, II, *M. F. Barrus* 5; Haina, Oct. 23, 1925, *R. Ciferri*.

Reported by Ciferri and Fragoso (Bol. R. Soc. Esp. Hist. Nat. 26: 330) as *Puccinia insueta* Wint. This name was founded on a rust from Brazil on some Malpighiaceae. The description given for the Brazilian specimen is very similar to *Puccinia inflata* and it is entirely possible that the two may be the same.

46. PUCCINIA INSULANA H. S. Jackson, Bot. Gaz. 65: 296. 1918. (*Bullaria*, N. Am. Fl. 7: 496, 1922.)

On *Vernonia racemosa* Delponte, Santiago, March 21, II, iii, 115.

Apparently a new host for this species, which heretofore has been known from Porto Rico, St. Croix, Jamaica, Guatemala, and Antigua. The teliospores are long and smooth with hyaline papillae over the germ pores.

47. PUCCINIA INVAGINATA Arth. & Johnston, Mem. Torrey Club 17: 146. 1918. (*Bullaria*, N. Am. Fl. 7: 488, 1922.)

On *Gouania lupuloides* (L.) Urban, San Cristobal, March 28, II, 105; Azua, March 29, II, 75.

48. PUCCINIA LANTANAE Farl. Proc. Am. Acad. Sci. 18: 83. 1883. (*Micropuccinia*, N. Am. Fl. 7: 559, 1922.)

On *Priva lappulacea* (L.) Pers., Bajabonico, March 20, 65; San Cristobal, March 12, 36; Los Ranchos, May 7, 1927, C. E. Chardon 378.

*Lantana involucrata* L., Barahona, Jan. 26, 1926, M. F. Barrus 7.

49. PUCCINIA LATERITIA Berk. & Curt. Jour. Acad. Sci. Phila. 2: 281. 1853. (*Micropuccinia*, N. Am. Fl. 7: 568, 1922.)

On *Borreria laevis* (Lam.) Griseb., San Pedro de Macoris, Feb. 1, 1926, M. F. Barrus 13.

50. PUCCINIA LEONOTIDIS (P. Henn.) Arth. Mycologia 7: 245. 1915. (*Dicaeoma*, N. Am. Fl. 7: 407, 1921.)

On *Leonotis nepetaefolia* (L.) R. Br., San Cristobal, March 12, II, 113; Monte Cristi, Aug. 12, 1918, John A. Stevenson 7001.

This species has usually been referred by American authors to *Puccinia Leonotidis* (P. Henn.) Arth., the type of which is from South Africa. While it seems probable that the American and African specimens are the same and that this is the proper name to be used, there is a confusion regarding the teliospores. Arthur in the N. Am. Fl. 7: 407 (1921) gives a description of the telial stage which is evidently from African material based on observations by Hennings (see Mycologia 7: 245, 1915). The teliospores are described as ellipsoid,  $18-23 \times 25-32 \mu$ . Fragoso and Ciferri in Bol. R. Soc. Esp. Hist. Nat. 26: 248 (1926) describe a new species, *Puccinia dominicana* Frag. & Cif., on *Leonotis nepetaefolia*, collected in Moca, Santo Domingo, Jan. 23, 1926, by J. Beccan. The urediniospores seem to be the characteristic ones found on this host. They describe teliospores as subfusoid,  $18-22$  by  $60-90 \mu$ , and their species is founded largely on the difference between these teliospores and those described by Hennings. I am inclined to think that we are dealing here with only one species on *Leonotis* as is indicated so strongly by the urediniospores and that there is an error either in one case or the other regarding the teliospores.

The Stevenson collection is reported in the N. Am. Fl.



51. PUCCINIA LEVIS (Sacc. & Bizz.) Magn. Ber. Deuts. Bot. Ges. 9: 190. 1891. (*Dicaeoma*, N. Am. Fl. 7: 286, 1920; 774, 1926.)

On *Panicum fasciculatum* Swartz, San Pedro de Macoris, Feb. 1, II, M. F. Barrus; San Cristobal, July, 1921, II, J. A. Faris (from phanerogamic specimen); Los Ranchos, May 7, 1927, II, C. E. Chardon 379.

*Panicum maximum* Jacq., Haina, June 26, 1925, R. Ciferri 830.

Reported on *Panicum maximum* by Fragoso and Ciferri (Bol. R. Soc. Esp. Hist. Nat. 25: 357, 1925) as *Puccinia Panici* Diet.

52. PUCCINIA MALVACEARUM Bertero; Mont. in C. Gay, Fl. Chile 8: 43. 1852. (*Micropuccinia*, N. Am. Fl. 7: 542, 1922.)

On *Malvastrum corchorifolium* (Desv.) Britton, Bajabonico, March 23, 76.

*Malvastrum coromandelianum* (L.) Garcke, Bajabonico, March 23, 63; La Vega-Moca road, March 20, 33; road Moca to Salcedo, May 7, 1927, C. E. Chardon 358.

53. PUCCINIA MEDELLINENSIS Mayor, Mem. Soc. Sci. Nat. 5: 497. 1913. (*Dicaeoma*, N. Am. Fl. 7: 408, 1921.)

On *Hyptis suaveolens* Poir. (*Mesosphaerum suaveolens* (L.) Kuntze), San Pedro de Macoris, Jan. 30, 1926, II, M. F. Barrus 12, 15.

54. PUCCINIA MELAMPODII Diet. & Holw.; Holw. Bot. Gaz. 24: 32. 1897. (*Micropuccinia*, N. Am. Fl. 7: 581, 1922.)

On *Synedrella nodiflora* (L.) Gaertn., Santiago, March 20, 1926, III, 122; Bajabonico, March 23, 100; river near Bonao, May 4, 1927, C. E. Chardon 343.

*Eleutheranthera ruderalis* (Sw.) Sch.-Bip., Haina, March 11, 99; Barahona, June, 1910, Pater Fuertes Phan. no. 174.

The Fuertes collection is the basis of the report in the N. Am. Fl.

55. PUCCINIA OBLIQUA Berk. & Curt. Jour. Linn. Soc. 10: 356. 1869. (*Micropuccinia*, N. Am. Fl. 7: 555, 1922.)

On *Funastrum clausum* (Jacq.) Schlecht., San Cristobal, March 14, 53.

56. PUCCINIA PALLESCENS Arth. Bull. Torrey Club 46: 111. 1919. (*Dicaeoma*, N. Am. Fl. 7: 278, 1920.)

On *Zea Mays* L., La Vega-Moca road, March 20, II, 116; Bajabonico, March 25, II, 87; La Vega, May 4, 1927, II, C. E. Chardon 348.

The Kern and Toro collection, No. 87, has both *Puccinia pallescens* and *Puccinia Sorghi*.

57. PUCCINIA PSIDII Wint. Hedwigia 23: 171. 1884. (*Bullaria*, N. Am. Fl. 7: 488, 1922.)

On *Jambos Jambos* (L.) Millsp., river near Bonao, May 4, 1927, C. E. Chardon 344.

58. PUCCINIA PURPUREA Cooke, Grevillea 5: 15. 1876. (*Dicaeoma*, N. Am. Fl. 7: 284, 1920; 774, 1926.)

On *Holcus halepensis* L., Haina, March 30, II, 72.

*Holcus Sorghum* L. (*Andropogon Sorghum sudanensis* Piper), San Francisco de Macoris, Aug. 18, 1926, R. Ciferri.

Ciferri and Fragoso (Bol. R. Soc. Esp. Hist. Nat. 26: 470, 1926) report *Uromyces Clignyi* Pat. & Har. Jour. de Bot. 14: 237, 1900, on *Holcus Sorghum*, the collection cited above. They found urediniospores only. Since the urediniospores of *U. Clignyi* are very similar to those of *Puccinia purpurea* in size, markings, and pores and since the usual rust on this host is *Puccinia purpurea*, I believe the weight of evidence favors the disposition here made. I have not had opportunity to examine the specimen collected by Dr. Ciferri.

59. PUCCINIA RIVINAE (Berk. & Curt.) Speg. Anal. Mus. Nac. Buenos Aires 19: 304. 1909. (*Dicaeoma*, N. Am. Fl. 7: 388, 1920.)

On *Trichostigma octandrum* (L.) H. Walt., Salcedo, May 7, 1927, II, C. E. Chardon 366.

60. PUCCINIA SCLERHICOLA Arth. Mycologia 7: 232. 1915.  
(*Dicaeoma*, N. Am. Fl. 7: 350, 1920.)  
On *Scleria secans* (L.) Urban, Bonao, March 16, II, iii, 19.
61. PUCCINIA SORGHII Schw. Trans. Am. Phil. Soc. II. 4: 295.  
1832. (*Dicaeoma*, N. Am. Fl. 7: 277, 1920.)  
On *Zea Mays* L., Bajabonico, March 25, II, 87.
62. PUCCINIA TUBULOSA (Pat. & Gaill.) Arth. Am. Jour. Bot. 5:  
464. 1918. (*Dicaeoma*, N. Am. Fl. 7: 288, 1920.)  
On *Paspalum conjugatum* Berg., Jayabo road to San Francisco  
de Macoris, May 7, 1927, II, C. E. Chardon 375.  
*Paspalum plicatulum* Michx., Santo Domingo, March 27,  
II, 26.  
*Syntherisma sanguinalis* (L.) Dulac., Bajabonico, March 23,  
ii, 68.  
*Valota insularis* (L.) Chase, Santiago, March 20, II, 121.
63. PUCCINIA URBANIANA P. Henn. Hedwigia 37: 278. 1898.  
(*Micropuccinia*, N. Am. Fl. 7: 558, 1922.)  
On *Valerianodes jamaicense* (L.) Kuntze, La Vega-Moca road,  
March 20, III, 117; Haina, March 11, 108; San Cris-  
tobal, March 12, 95; San Pedro de Macoris, Jan. 21,  
1926, M. F. Barrus 9.  
? *Cornutia pyramidata* L., San Cristobal, March 14, 60;  
Puerto Plata, March 24, 58.
64. AECIDIUM TOURNEFORTIAE P. Henn. Hedwigia 34: 338.  
1895. (*Aecidium*, N. Am. Fl. 7: 634, 1924.)  
On *Tournefortia hirsutissima* L., San Cristobal, March 14, 55.
65. UREDO ARTOCARPI Berk. & Br. Jour. Linn. Soc. 14: 93.  
1873. (*Physopella*, N. Am. Fl. 7: 103, 1907.)  
On *Artocarpus communis* Forst., La Vega, March 19, II, 124.
66. UREDO COCCOLOBAE P. Henn. Hedwigia 35: 253. 1896.  
(*Uredo*, N. Am. Fl. 7: 609, 1924.)  
On *Coccolobis uvifera* (L.) Jacq., Haina, March 30, II, 104.

67. UREDO CUPHEAE P. Henn. Hedwigia 34: 99. 1895. (*Uredo*, N. Am. Fl. 7: 614, 1924.)

On *Parsonsia Parsonsia* (L.) Britton, Bajabonico, March 23, 80.

68. UREDO HAMELIAE Arth. Mycologia 8: 23. 1916. (*Uredo*, N. Am. Fl. 7: 617, 1924.)

On *Hamelia erecta* Jacq., Puerto Plata, March 24, 81.

69. UREDO IGNAVA Arth. Bull. Torrey Club 46: 121. 1919. (*Dicaeoma*, N. Am. Fl. 7: 341, 1920; 783, 1926.)

On *Bambos vulgaris* Schrad., San Pedro de Macoris, March 10, 109.

70. UREDO JATROPHICOLA Arth. Mycologia 7: 331. 1915. (*Uredo*, N. Am. Fl. 7: 613, 1924.)

On *Adenoropium gossypifolium* (L.) Pohl. (*Jatropha gossypifolia* L.), Los Matas, March 28, 106; San Cristobal, March 12, 94; Sanchez, Aug. 4, 1918, *John A. Stevenson* 7023; La Vega, Aug. 7, 1918, *John A. Stevenson* 7063. *Curcas Curcas* (L.) Britton & Millsp., Haina, March 11, 98; Puerto Plata, March 24, 85.

Previously reported both by Fragoso and Ciferri and the N. Am. Fl.

71. UREDO SAPOTAE Arth. & Johnston, Mem. Torrey Club 17: 169. 1918. (*Uredo*, N. Am. Fl. 7: 615, 1924.)

On *Sapota Achras* Mill., Santiago, March 20, 34.

72. *Uredo Toroiana* sp. nov.

On *Vernonia cinerea* (L.) Less., Puerto Plata, March 24, 1926, II, *Kern & Toro* 84 (type); San Pedro de Macoris, Feb. 1, *M. F. Barrus*.

II. Uredinia hypophyllous, scattered or somewhat crowded, roundish, 0.3–0.4 mm. in diameter, early naked, pulverulent, yellowish-brown; paraphyses peripheral, numerous, incurved, clavate, often much bent, septate,  $14-18 \times 40-80 \mu$ , the wall colorless, about  $3 \mu$  thick, smooth; urediniospores ellipsoid or obovoid,  $19-26 \times 22-30 \mu$ , the wall colorless,  $1.5 \mu$  thick, moderately and evenly echinulate, the pores obscure.

A very interesting rust which in the field had the general appearance of a *Coleosporium*. A microscopical examination soon

showed that this was not the case. The incurved jointed paraphyses make this species distinctive. In this regard and in host-relationship there is a close parallel to *Uredo Vernoniae-hookerianae* Petch (Ann. R. Bot. Gard. Peradeniya, III, 7: 213, 219, 1917) from Ceylon. It is most certainly the same type of rust and the two undoubtedly belong to the same genus. Differences in the sori, in the size of the spores and paraphyses, and in the walls of the paraphyses, have prevented me from considering these two to be the same species. The name is in honor of my associate.

73. *Uredo bullula* sp. nov.

On *Eupatorium* sp., Santiago, March 22, 1926, II, Kern & Toro 8.

II. Uredinia hypophyllous, scattered, roundish, pustular, 0.3–1 mm. across, long covered by the brown overarching epidermis, finally opening by an irregular central break; paraphyses none; urediniospores broadly ellipsoid or obovoid, sometimes compressed and irregular,  $23\text{--}26 \times 27\text{--}32 \mu$ , the wall cinnamon-brown,  $1.5 \mu$  thick, moderately and evenly echinulate, the pores 2, opposite and equatorial.

A species distinctive on account of the firm blister-like sori. In this regard it is quite different from an ordinary pulverulent form like *Uredo eupatoriicola* P. Henn. from Brazil. It is somewhat more like *Uredo suspecta* Jackson & Holway, from Costa Rica, but the spores of that species are larger, rougher, thicker-walled, and the sori are not bullate.

SPECIES PREVIOUSLY REPORTED FROM SANTO DOMINGO, NOT  
IN THE FOREGOING LIST

74. *COLEOSPORIUM DOMINGENSIS* (Berk.) Arth. Am. Jour. Bot.  
5: 329. 1918.

*Uredo domingensis* Berk. Ann. Mag. Nat. Hist. II. 9: 200.  
1852.

*Coleosporium Plumierae* Pat. Bull. Soc. Myc. Fr. 18: 178.  
1902. (N. Am. Fl. 7: 87, 1907; 652, 1924.)

On *Plumiera rubra* L. Ex-Herb. Kew, No. 78, probably part of  
type (locality unknown—collector unknown—published  
in a paper by Berkeley, "Enumeration of some fungi

from Santo Domingo." See Arth. Am. Jour. Bot. 5: 329. 1819).

This rust is known also from Bahamas, Guatemala, and Panama.

75. PHAKOPSORA FENESTRALA Arth. Bull. Torrey Club 44: 508. 1917. (N. Am. Fl. 7: 674, 1925.)

On *Phyllanthus grandifolius* L., La Romana, Dec. 1-3, 1909, *N. Taylor* 365.

Reported in the N. Am. Fl.

76. PHAKOPSORA MEIBOMIAE Arth. Bull. Torrey Club 44: 509. 1917. (N. Am. Fl. 7: 673, 1925.)

On *Meibomia tortuosa* (Sw.) Kuntze, prope Maniel de Ocoa, October, 1910, *H. von Turckheim Phan. no. 3656*.

Reported in the N. Am. Fl.

77. TRANZSCHELIA PUNCTATA (Pers.) Arth. Résult. Sci. Congr. Bot. Vienne 340. 1906. (N. Am. Fl. 7: 151, 1907; 720, 1926.)

On *Prunus spinosa* L. (cult.), Moca, January, 1927, II, *R. Ciferri*.

Reported by Ciferri and Fragoso (Bol. R. Soc. Esp. Hist. Nat. 27: 267, 1927) as *Puccinia Pruni-spinosae* Pers. which is a synonym of the name here used.

78. UROMYCES COMEDENS Syd. Monog. Ured. 2: 37. 1910.

On *Jasminum pubescens* Willd., Haina, April 18, 1925, *R. Ciferri* 851.

This species is closely related to *Uromyces Hobsoni* Vize (Grevillea 4: 115, 1876) from which it differs in having larger aeciospores and in having its teliospores built into the aecial cups which retain their shape. *U. Hobsoni* is on *Jasminum grandiflorum*. Sydow does not describe pycnia for *U. comedens* but Fragoso and Ciferri (Bol. R. Soc. Esp. Hist. Nat. 25: 356, 1925.) do. I have a specimen but it is too fragmentary for me to decide the question of relationship between these two rusts.

79. UROMYCES TRICHOLAENAE Frag. & Cif. Bol. R. Soc. Esp. Hist. Nat. 25: 357. 1925.

On *Tricholaena rosea* Nees, Haina, June 28, 1925, *R. Ciferri* 90.

Reported by Fragoso and Ciferri, Bol. R. Soc. Esp. Hist. Nat. 25: 357 (1925), as a new species *ad interim*.

The rust to be expected on this host is *Puccinia levis*. In the part of the type available only urediniospores are found which agree well with *P. levis*. Fragoso and Ciferri describe 1-celled teliospores. My specimen is too fragmentary to lead to a decision which would satisfactorily determine its standing.

80. PUCCINIA ARTHURELLA Trotter in Sacc. Syll. Fung. 23: 694. 1925. *Puccinia proximella* Arth. Bull. Torrey Club 47: 471. 1920. (*Dicaeoma*, N. Am. Fl. 7: 439, 1921.)

On *Brachyramphus intybaceus* (Jacq.) DC. (*Lactuca intybacea* Jacq.), Azua, March 18, 1913, *Rose, Fitch & Russell Phan. no. 4015*.

Reported in the N. Am. Fl.

81. PUCCINIA PLUCHEAE (Syd.) Arth. Bull. Torrey Club 49: 194. 1922. (*Dicaeoma*, N. Am. Fl. 7: 793, 1926.)

On *Pluchea purpurascens* (Sw.) DC., San Pedro de Macoris, March 31, 1913, *Rose, Fitch & Russell Phan. no. 4294*.

Reported in the N. Am. Flora.

82. PUCCINIA POLYSORA Underw. Bull. Torrey Club 24: 86. 1897. (*Dicaeoma*, N. Am. Fl. 7: 279, 1920.)

On *Tripsacum dactyloides* L., Constanza, May, 1910, *H. von Turckheim Phan. no. 3320*.

Reported in the N. Am. Fl.

83. PUCCINIA XANTHII Schw. Schr. Nat. Ges. Leipzig 1: 73. 1822. (*Micropuccinia*, N. Am. Fl. 7: 571, 1922.)

On *Xanthium chinense* Mill., Sanchez, Apr. 2, 1913, *Rose, Fitch & Russell Phan. no. 4351*.

Reported in the N. Am. Fl.

84. *AECIDIUM DOMINICANUM* Frag. & Cif. Bol. R. Soc. Esp. Hist. Nat. 26: 249. 1926.

On *Ipomoea* sp., Haina, Nov. 20, 1925, *R. Ciferri*.

Reported by Ciferri and Fragoso.

85. *UREDIO BIXAE* Arth. Mycologia 7: 327. 1915. (*Uredo*, N. Am. Fl. 7: 613, 1924.)

On *Bixa orellana* L., Haina, June, 1925, *R. Ciferri*.

Previously known only from Porto Rico.

- Reported from Santo Domingo by Fragoso and Ciferri, Bol. R. Soc. Esp. Hist. Nat. 25: 443. 1925.

86. *UREDIO EICHORNIAE* Frag. & Cif. Bol. R. Soc. Esp. Hist. Nat. 27: 69. 1927.

On *Piaropus crassipes* (Mart.) Britton (*Eichornia crassipes* Solms), Haina, February, 1926, *R. Ciferri*.

The authors say they found no rust reported on this host and therefore describe this as a new species. The urediniospores are  $21-24 \times 22-26 \mu$ , the wall  $3.5 \mu$  thick, sparsely verrucose, with 3-5 pores; paraphyses are numerous, linear or clavate, and incurved. According to the illustration the pores are scattered. I have not seen a specimen.

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*Tripsacum dactyloides*  
*Puccinia polysora*  
*Triumfetta semitriloba*  
*Triumfetta* sp.  
*Puccinosira pallidula*  
  
*Valerianodes cayennense*  
*Endophyllum Stachytarphetae*  
*Valerianodes jamaicense*  
*Puccinia Urbaniana*  
*Valota insularis*  
*Puccinia tubulosa*  
*Vernonia cinerea*  
*Uredo Toroiana*  
*Vernonia racemosa*  
*Puccinia insulana*  
*Vernonia Sprengeliana*  
*Puccinia fuscella*  
*Vigna repens*  
*Uromyces appendiculatus*  
  
*Wedelia Ehrenbergii*  
*Wedelia reticulata*  
*Uromyces piauihyensis*  
  
*Xanthium chinense*  
*Puccinia Xanthii*  
  
*Zea Mays*  
*Puccinia pallescens*  
*Puccinia Sorghi*

## NOTES ON THE SYNONYMY OF SOME SPECIES OF HYPOXYLON

C. L. SHEAR

For many years the writer has been making more or less desultory studies of *Hypoxylon*. During this period we have had an opportunity to examine the types or authentic material of most of the older species described by European and American authors. These studies have shown that there is considerable error and confusion in the interpretation of the species. While the study of type material is helpful in giving us more exact knowledge of the character of the species, especially the spores, still it is necessary to have a broad field knowledge of the species and a large amount of material from different regions showing all stages and conditions of their development in order to reach dependable conclusions. A careful study is needed, especially of the conidial forms. Very little attention has ever been paid to these. The few observations and comparisons which have been made show that in some cases, at least, species which can scarcely be distinguished in their mature form show easily recognizable differences in their conidial stages.

The assumption that stromatic characters are constant and specific in this genus has led to many mistakes in the segregation and recognition of the species. How constant or reliable such characters may be in any species can only be determined by an examination of a large quantity of material collected in different localities and under different conditions. In *Hypoxylon coccineum*, for instance, the stromata are unusually constant in shape and other characters, varying mostly in size only. In *H. multiforme*, on the other hand, the stromata are very variable in shape, ranging from the common pulvinate form through all sorts of irregular forms to an effuse stroma similar to that of *H. rubiginosa*.

The same is true of spore characters. In some species the range of variation in the spores in size and shape is very slight

whereas in other species there is considerable range of variation, making it very difficult to determine the specific limitations.

Notes on the synonymy of the following species are given in alphabetical order.

*H. annulatum* (Schw.) Curt. = effuse form of *H. marginatum* (Schw.) Berk. q.v.

*H. atropurpureum* Fr. = *H. multiforme* Fr. q.v.

*H. atrorufum* Ellis & Ev. = *H. cohaerens* (Pers.) Fr. q.v.

*H. bifrons* De-Not. = *H. Sassafras* (Schw.) Curt. q.v.

*H. Blakei* Berk. & Curt. = *H. Morsei* Berk. & Curt. q.v.

*H. callostroma* (Schw.) Berk. = *H. Sassafras* (Schw.) Curt. q.v.

*H. caries* (Schw.) Sacc. = *H. serpens* (Pers.) Fr. q.v.

*H. Catalpae* (Schw.) Sacc. = *H. perforatum* (Schw.) Curt. q.v.

*H. COCCINEUM* Bull. = *H. enteromelum* (Schw.) Cooke = *H. Howeianum* Peck.

A specimen of *Sphaeria enteromela* from the herbarium of Schweinitz preserved in Michener's herbarium is a mere form of *H. coccineum*. The specimen consists of two pieces of bark, one apparently beech and the other chestnut. In the first the stromata are young; in the second they are nearly mature and are typical *coccineum*. Cooke, who examined specimens from Schweinitz in Berkeley's herbarium, says the spores are  $10 \times 3 \mu$ , which is the usual size for *coccineum*.

*H. Howeianum* of Peck, according to our measurements made from part of the type collection, has spores  $8-10 \times 4 \mu$ . Peck says this is allied to *H. fragiforme*, but is larger and has a punctate surface and smaller spores. He, however, gives no spore measurements. We adopt *H. coccineum* as the name for this species on the basis of its wide general usage and correct application, although older names have evidently been applied to it. This species we also regard as the type of the genus *Hypoxylon*.

*H. COHAERENS* (Pers.) Fr. = *H. turbinulatum* (Schw.) Ellis & Ev.  
= *H. atrorufum* Ellis & Ev.

The types of these two species in the herbaria of their authors show the same sized spores,  $9-12 \times 4-5 \mu$ , and have the typical form of this well-known and characteristic species. *H. atrorufum* was in a fresh, just maturing condition in which the stroma is

usually a very dark reddish color. Schweinitz's species is a mere form in which the stromata are a little more uniform and elevated than the type of *H. cohaerens*.

*H. colliculosum* (Schw.) = *H. serpens* (Pers.) Fr. q.v.

*H. decorticatum* (Schw.) Berk. = *H. perforatum* (Schw.) Curt. q.v.

*H. durissimum* (Schw.) Cooke = *H. perforatum* (Schw.) Curt.  
q.v. and not *H. marginatum* as per Ellis & Ev. N. A.  
Pyren. 640.

*H. enteromelum* (Schw.) Cooke = *H. coccineum* Bull. q.v.

*H. Howeianum* Pk. = *H. coccineum* Bull. q.v.

*H. MARGINATUM* (Schw.) = *H. annulatum* (Schw.) Curt.

The specimen of *Sphaeria marginata* No. 1176, in Schweinitz's mounted collection, has ascospores  $7.5-10 \times 4-5 \mu$ , mostly  $8 \times 4 \mu$ . *H. annulatum*, as shown by Schweinitz's original illustration and authentic specimens, is simply an effuse form of *marginatum* which sometimes shows more or less scattered perithecia, especially when found on decorticated wood. The spores and other characters are practically the same. Schweinitz apparently concluded that these two names were synonyms, as he did not include *annulatum* in his North American Fungi. Although the specific name *annulatum* (1825) has priority over *marginatum* (1832), the name *marginatum* should be adopted for the species on account of its wide general use for this common species and because it was applied to the most common form of the species.

The name *H. annulatum*, as used by Montagne and attributed to Fries, probably refers to another species. *Sph. marginata* Fr. (1828) is, according to the original description, a *Nummularia*, probably *N. discreta*, according to Saccardo and Ellis.

*H. MULTIFORME* Fr. = *H. granulosum* Bull. = *Sphaeria rubiformis*  
Pers. = *H. atropurpureum* Fr. = *H. transversum* (Schw.)  
Sacc.

Authentic specimens of Fries have spores  $10-12 \times 4-5 \mu$ . *H. granulosum* is accepted as a synonym on the basis of Bulliard's illustration and the authority of Persoon, Fries and others. Persoon's type of *Sphaeria rubiformis* agrees entirely with this species. *H. atropurpureum* has not before been considered a

synonym of this species but an examination of authentic specimens of Fries issued in his "Scleromycetes Suecica" shows that this is only an effuse condition of *multiforme*, it having all the other stromatic and spore characters. Intergrading forms are frequently found as to the shape of the stroma. *H. transversum* (Schw.) Sacc. differs only from typical *multiforme* in that the stromata arise in transverse cracks in the bark of birch, the host upon which it is most commonly found. Spores and all the other characters of Schweinitz's specimens agree entirely with this species.

*H. PERFORATUM* (Schw.) Curt. = *H. Catalpae* (Schw.) Sacc. =  
*H. decorticatum* (Schw.) Berk. = *H. durissimum* (Schw.)  
Cooke.

*H. perforatum* is a rather variable species, especially as to size, shape and color of the stromata. When occurring on bark the stromata are usually small, irregular, pulvinate to subglobose, and when occurring on decorticated wood they are frequently effuse and very similar to *H. rubiginosum*. The spores according to measurements made from Schweinitz's original collection, apparently on *Liquidambar*, range from  $11-15 \times 5-7.5 \mu$ , mostly  $11-12 \times 5-6 \mu$ . Schweinitz's specimens of *H. Catalpae* show the effuse form of the stroma with spores mostly  $11 \times 5.5 \mu$ . His specimen of *H. decorticatum* is the effuse form on decorticate wood with ascospores mostly  $11 \times 5 \mu$  and is not a synonym of *H. marginatum* as given by Ellis and Everhart. This species is very close to *H. rubiginosum* Pers. and it may be that intergrading forms occur. Much more thorough and careful study of *H. rubiginosum* in all its forms and conditions is needed to define satisfactorily its specific limits and synonymy.

*H. SASSAFRAS* (Schw.) Curt. = *H. callostroma* (Schw.) Berk. =  
*H. bifrons* De-Not. = *Rosellinia Linderae* Peck. = *Rosellinia prinicola* Berk. & Curt.

The spores of Schweinitz's specimens of *H. Sassafras* range from  $8-11 \times 3-5 \mu$ . This species is very variable in the aggregation and arrangement of the perithecia which are rather frequently scattered and sometimes separate. This has led to the description of the latter forms under the genus *Rosellinia*. An examina-

tion of the type or authentic material of the synonyms given shows that they correspond in spore size and in the character of the perithecia and the yellow layer surrounding them. The rather unusual characteristic of this species seems to be its host restriction, being found at present only on hosts belonging to the Lauraceae. Berkeley and Curtis' *R. prinicola* was so named on account of an incorrect identification of the host. The type is clearly on *Lindera* and not on *Prinus*. *Sphaeria corticata* Pers. in Herb. is the same plant. This name was never published apparently. The specimen was sent from Pennsylvania by Muhl-enberg and is on *Lindera*.

*H. SERPENS* (Pers.) Fr. = *H. caries* (Schw.) Sacc.

The spores in Persoon's type are  $11-15 \times 5.5-6.5 \mu$ , mostly  $12.5 \mu$  long. In Schweinitz's type the spores are  $9-14 \times 5-6 \mu$ . The black line penetrating the wood below the stroma in this species is supposed to be characteristic. It, however, does not appear from our observations to be a constant character, but seems to depend upon the condition of the wood upon which it is growing.

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## DARK-FIELD MICROSCOPY IN THE STUDY OF FUNGI

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(WITH PLATE 13)

During a monographic study of the monilioid species of *Sclerotinia*, by the senior writer, it became desirable to photograph certain microscopic structures such as asci, ascospores and paraphyses. In all species of this group studied, these structures appear hyaline or nearly so and for this reason present a problem quite distinct from that of similar dark colored structures. Where color is present in the object contrast is readily secured with the bright-field microscope. Hyaline objects are not so readily visible, and to enhance their visibility it is necessary to greatly reduce the amount of light transmitted through them. Even by a reduction of light in the bright-field, certain details of hyaline objects are not visible or are not so distinct as in the dark-field. The adaptability of dark-field illumination to the study of living hyaline unstained material as is illustrated in this paper is one of its advantages over the bright-field. Beck and Gage (1925:428) firmly maintain not only that the dark-field microscope makes objects visible but also that the maximum resolution may be obtained. In fact Beck is quoted (Gage, 1925:428) as saying, "Anything that can be resolved by transmitted illumination can be resolved by dark-ground illumination, and in general with much greater brilliancy, because of the increased contrast between different parts of the structure."

So far as the writers are aware, dark-field microscopy has not been applied to the study and photography of the morphological structures of the higher fungi. Atkinson (1900) in photographing gross characters of the Agaricaceae and other fungi, Dickson and Fisher (1923) in photographing spore-clouds arising from dis-

<sup>1</sup> For the critical reading of the manuscript, the writers are indebted to Professor H. H. Whetzel, Dr. H. M. Fitzpatrick, Dr. L. M. Massey and Dr. S. H. Gage.



charging apothecia, and others have made use of the *black background* to aid in resolution. Noguchi (1919) used dark-field microscopy for the study and photography of the organism causing yellow fever, and Park and Williams (1920: 71-72) report the use of the dark-field in the study of such organisms as *Treponema pallidum* and the flagella on certain bacteria. Gaidukov (1910) has given a very complete review of the uses to which dark-field illumination has been put in the biological field, including observations on bacteria, myxomycetes, flagellates, beer yeasts, algae, etc. The writers have made use of dark-field microscopy in the study of the morphology and in connection with the photography of several North American species of *Sclerotinia*. Fresh living material has been studied in all of the dark-field work. We were most fortunate in receiving suggestions and the loan of some of the apparatus used in the work, from Doctor Simon H. Gage.

The principles and details of application of dark-field illumination have been ably discussed and illustrated by Gage (1925) in the fourteenth revision of his text, "The Microscope," and by Chamot (1921). Since in dark-field illumination the rays pass through the condenser in a hollow cone, sufficiently oblique as not to pass directly into the objective, the object, which in our case consists of spores, ungerminated or germinated, asci and paraphyses, appears light in a dark background. While the reader is referred to Gage (1925), Chamot (1921), Barnard and Welch (1925) or other authorities on dark-field illumination and photomicrography for details, it may be worth while to mention some of the essentials which we have found necessary for success in its use with fungi:

1. The objects must be in a medium of a refractive index different from themselves. We have found distilled water free from crystals or other particles satisfactory for this purpose. The greater the difference between the refractive index of the object and the medium in which it is suspended the brighter will be the appearance of the object. The age of the fungous object used seems to affect somewhat its ability to refract light, for asci which were either quite immature or from apothecia far beyond their prime were less satisfactory. All experiments with thin films of agar, as media for mounting the objects upon thin slides to con-

form to the limits of thickness required by the dark-field condenser, proved unsatisfactory. The agar film introduced more difficulties in connection with the lighting, and is more tedious, and was abandoned in favor of the water medium.

2. The objects must be scattered in the dark-field, for if there are no intervening spaces the entire field will be bright, and the details not being distinct cannot be studied advantageously. Therefore, in making mounts care should be taken to properly tease apart the asci and paraphyses, and have these objects uniformly dispersed throughout. When ascospores are to be photographed, apothecia attached to the top of a deep petri dish should be allowed to discharge them directly into a small drop of distilled water on a slide of the proper thickness. If the spore suspension is found to be too concentrated, it should be diluted by transferring to other drops of distilled water on other slides. By allowing the spores to discharge into the water, it will be found easier to keep out foreign particles or bits of tissue from the apothecium. Such debris will also reflect light and thus make the spores less distinct.

3. Cover-glasses and microscope slides of the proper thickness must be used. This thickness will vary with the refracting condenser used. Most condensers have the equivalent focus and working distance marked on them, and this should be consulted, and slides and cover-glasses selected accordingly by use of micrometer calipers. If this is done, the focus of the condenser is brought to the upper surface of the microscope slide where the object is situated, and thus the most satisfactory illumination secured. The dark-field condenser used by the writers was labelled with a slide thickness of 1.45–1.55 mm. Slides ranging between these extremes, preferably those of 1.50 mm., were used. Thin square cover-glasses, 0.15–0.18 mm. in thickness, were employed in order to allow for the proper working distance for the objective used.

4. The object should be mounted in as thin a film of water as possible. This is to insure the greater probability of finding the entire object in the same focal plane. Elongate objects, such as asci, will cause more difficulty in this respect than shorter objects such as spores. Success depends as much upon this one condition

as upon any other, and next to the lighting problem, is the greatest limiting factor in securing satisfactory results. If the thickness of the slide recommended by the maker of the condenser should not prove satisfactory, it may be necessary to determine the thickness best suited to the particular apparatus. A method for accomplishing this is given by Gage (1925: 443-445).

5. The mount must be sealed by a thin layer of shellac or gold-size applied with camel's hair brush about the edge of the cover-glass. This attaches the cover firmly to the slide and prevents evaporation with the resulting movement of the object during exposure.

6. Slides and cover-glasses must be thoroughly cleaned. We are indebted to Dr. Gage for suggesting the method, which he has since published (1925: 315-316), and which has proved very satisfactory. This is the use of Bon Ami emulsion (5 gr. stirred up in 100 cc. of water) into which the slides are placed and stirred, and then taken out one by one and set up on end on blotting paper to dry. The thoroughly dried slides are kept in a covered container and, when needed, wiped well with a piece of fresh gauze. Cover-glasses are treated in the same way.

7. The arrangement and source of light is of prime importance. A dimly lighted room should be chosen in which to work, the curtains drawn, when necessary, to eliminate direct sunlight. The source of light is very important. Direct sunlight is said to be the best, but it is not so satisfactory for continuous observation. Next to sunlight the arc lamp gives the most brilliant light, but we have found, under our set of conditions, that the 6-volt head-light lamp, described by Gage (1925: 447), is most suitable. In fact, for part of the work, we are indebted to Dr. Gage for the loan of a lamp of this type. When the Bausch and Lomb euscope was used, the lighting system supplied with it also proved satisfactory. The ideal to be sought in setting up and adjusting the apparatus is a perfectly black field, allowing only the light reflected from the object to pass into the microscope. Tests have shown that there is a certain amount of adventitious light entering the microscope, tending to render the background grayish, and that this increases with the brilliancy of the illumination even when the condenser, the microscope slide, the mirror,

etc., are most favorably arranged. We have found it desirable, therefore, in some of the work to lessen the intensity of the light by placing a sheet of ground-glass in front of the condenser mirror in the path of the light from the lamp, as suggested by Gage (1925:453). The closer the ground-glass is set to the microscope mirror, the more brilliant the light. A ground-glass whose surface had been oiled and from which the excess of oil had been rubbed was also used to some extent to avoid subduing the light too much. Also the field may be made darker by closing the iris more or less when a paraboloid condenser is employed, although this is not desirable with a cardioid condenser, for the most desirable part of the light would then be cut off. The lowering of the intensity of the light, as described above, will necessitate the increase of time exposure in making the photograph. With some objects where movement from the plane in which they have been focused for photographing or rotation motion is evident, it may be more desirable to allow more light and give shorter exposure. The time of exposure will vary, also, according to the distance of the source of light from the substage mirror.

8. The numerical aperture of the objective must be less than that of the dark-field condenser in order to secure a dark-field. Unless one is in the possession of immersion objectives of correct numerical aperture, designed primarily for dark-field microscopy, it will be necessary when using immersion objectives to place a funnel-stop into the back of the objective in order to block out direct rays which would otherwise give rise to a bright field.

9. In connection with the lighting it is important to properly center and adjust the substage dark-field condenser. The condenser should be raised to a level with the upper face of the microscope stage in order to be close to the slide. A drop of immersion oil must be placed on the top of the condenser. When the slide is in place, there will be a film of oil between the top of the condenser and the underside of the slide. This is true when either a dry or an oil-immersion objective is used. The small ring in the middle of the upper face of the dark-field condenser will aid in centering the condenser exactly in the field and this can be adjusted and maintained by means of the centering screws on the condenser. This important detail, as well as the method of

focusing the dark-field microscope with immersion objectives, is more fully treated by Gage (1925: 455-457).

If there is difficulty in securing a satisfactory dark background, it may be because (1) of an improper adjustment of the diaphragm on the stage or in the substage condenser; (2) of an aperture in the immersion objective unsuitable for the condenser resulting in direct light entering the objective; or (3) of air bubbles in the immersion-oil either between the condenser and the microscope slide or between the immersion objective and the upper surface of the cover-glass.

10. In selecting a room and the support for setting up the dark-field apparatus, consideration should be given to the possibility of vibrations affecting the final result of the exposures. The danger of trouble from this source increases with the higher objectives and the longer time of exposure.

In the first photographs of fungous structures attempted by the writers, a compound microscope with ordinary 4 mm. and 1.9 mm. objectives and a 10  $\times$  ocular was employed. Oculars and objectives of various magnifying powers were tested. Where oil-immersion objectives were used it was necessary to insert a funnel-stop in the back of the objectives to lessen the numerical aperture. The condenser used was a Bausch and Lomb paraboloid dark-ground illuminator. An ordinary vertical photographic camera (Bausch and Lomb) was adjusted to the ocular of the compound microscope after the object was in focus. The focus was then verified on the ground-glass of the camera above, the plate inserted, and the exposure made. Satisfactory results were obtained.

In the spring of 1925, the Bausch and Lomb Optical Company of Rochester, New York, placed at the disposal of the Department of Plant Pathology, Cornell University, for trial, one of their eusscopes. The writers were fortunate in being able to use this apparatus in studying several species of *Sclerotinia*. The euscope made it possible to study and photograph asci, ascospores, and paraphyses with greater ease and speed than was possible with the vertical camera. It may be helpful at this point to record a typical arrangement of apparatus and the operation as conducted by us in photographing with the dark-field.

(a) For photographing asci: Ocular Leitz Periplan 10  $\times$ ; objective achromatic 4 mm.; tube length set at 175 mm.; Bausch and Lomb paraboloid dark-field illuminator; Bausch and Lomb euscope with accompanying lighting system; plate used: orthochromatic cut film (4  $\times$  5); microscope slide: thickness between 1.45 mm. and 1.55 mm. (used 1.51 mm., also 1.52 and 1.53 with this condenser); mounting fluid: object in suspension in distilled water and sealed with thin shellac; exposure: a ground-glass was placed in front of the microscope mirror and exposures were made of 4, 8, 10, and 20 seconds. Eight seconds proved to be most satisfactory.

(b) For photographing ascospores: The apparatus was the same as above with the following exceptions: Ocular 8  $\times$  periplan (Leitz) and 5  $\times$  ocular; the 5  $\times$  ocular was found to be most satisfactory; objective: achromatic 1.9 oil immersion; mount was made by allowing apothecia to discharge spores directly into a drop of distilled water upon the slide; the thin mount was sealed with shellac; exposure: one second without ground-glass in front of mirror; four seconds with ground-glass seemed too long an exposure.

From our experiments with various oculars and objectives it was concluded (1) that a 3 mm. oil-immersion objective is more satisfactory than a 1.9 mm. or a 4 mm. dry objective since it throws a larger real image than the latter and yet results in less loss of light, and also has a smaller magnification and greater working distance than the former; (2) that increasing magnification with oculars alone (when using a 4 mm. dry objective) has limitations which make it impossible to produce as good results as with the 3 mm. oil-immersion with lower powered oculars.

When the negative from an exposure with the dark-field condenser is printed, the object photographed will appear white on a dark background. If this is successfully carried out, the result is very pleasing. However, a test was made to determine whether satisfactory results could be obtained by making a positive film from the negative and then by printing from the positive, to secure dark objects upon a white background. In cases in which it was tried, this also proved entirely satisfactory (PLATE 13, FIGS. *a* AND *d*), yet we are inclined, in most cases, to

favor the use of the original negative, which gives the dark-field effect. Results typical of the dark-field method of photographing are shown and explained in the accompanying plate (PLATE 13). All figures are made from the untouched films.

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#### EXPLANATION OF PLATE 13

Fig. a. Ascospores of *Sclerotinia fructicola* (Winter) Rehm taken with dark-field (paraboloid) condenser. Printed from the positive of Fig. b, instead of the negative. This gives the bright-field effect.

Fig. b. Same as Fig. a, except printed directly from the negative, thus giving the dark-field effect. Taken with a 4 mm. objective and 10 × periplan ocular. A ground-glass was placed in front of the microscope mirror and an exposure of 18 seconds made.

Fig. c. Ascospores of *Sclerotinia Amelanchieris* Reade germinating by the production of microconidia from each pole. Similar arrangement of apparatus as used in Fig. b.

Fig. d. Ascus of *Sclerotinia Vaccinii-corymbosi* Reade. Printed from positive of Fig. e.

Fig. e. Ascus of *Sclerotinia Vaccinii-corymbosi* Reade. Printed directly from the negative of Fig. d. Used 4 mm. objective and 10 × periplan ocular. A ground-glass was placed in front of mirror. Exposure 8 seconds.

Fig. f. Ascus of *Sclerotinia fructicola* (Winter) Rehm. Used 4 mm. objective and 5 × ocular.

Fig. g. Paraphysis of *Sclerotinia fructicola* (Winter) Rehm. Used 4 mm. objective and 10 × ocular.

Fig. h. Ascospores (one germinating by means of germ-tube) of *Sclerotinia fructicola* (Winter) Rehm. Used 4 mm. objective and 10 × ocular.

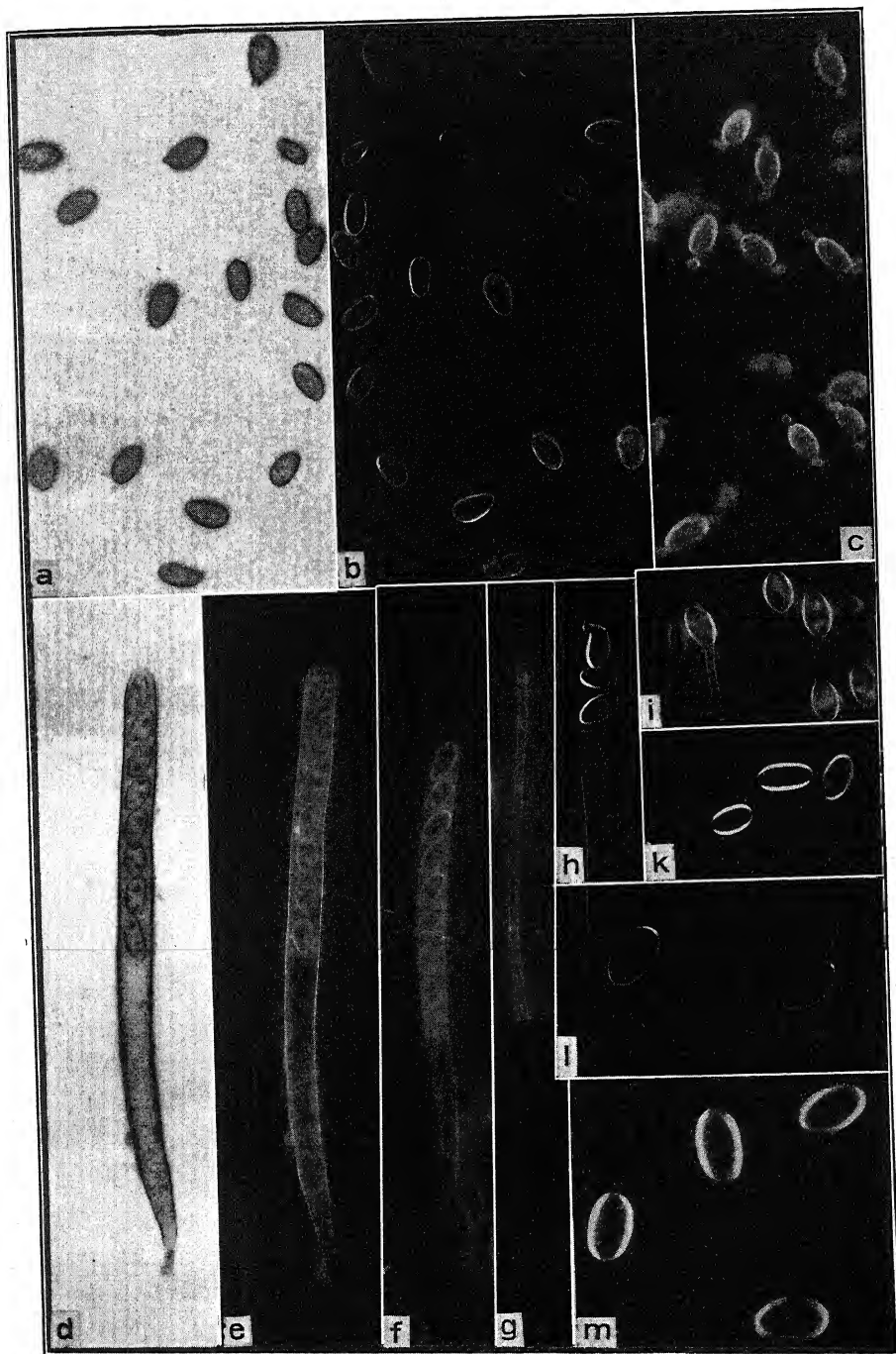
Fig. *i*. Ascospores of *Sclerotinia Vaccinii-corymbosi* Reade (one germinating by means of a germ-tube). Used 1.9 mm. oil-immersion objective and 5 × periplan ocular. Time 1 second without ground-glass in front of mirror.

Fig. *k*. Ascospores of *Sclerotinia Amelanchieris* Reade. Used 4 mm. objective and 10 × ocular.

Fig. *l*. Ascospores of *Sclerotinia Polycodii* Reade. Used 3 mm. oil-immersion objective and 10 × ocular.

Fig. *m*. Ascospores of *Sclerotinia Amelanchieris* Reade. Used 1.9 mm. oil-immersion objective and 10 × periplan ocular. Compare this figure with Figs. *k* and *l*.





DARK-FIELD MICROSCOPY



# A KEY TO THE KNOWN AECIAL FORMS OF COLEOSPORIUM OCCURRING IN THE UNITED STATES AND A LIST OF THE HOST SPECIES

GEORGE G. HEDGCOCK<sup>1</sup>

The determination of the aecial forms of *Coleosporium*, viz., the needle forms of *Peridermium*, on species of pine is often a difficult task, especially in the field. The writer has collected and named upwards of 3,000 specimens of this group in the United States. In arranging a key an effort is made to combine for the use of others some of the macroscopic characters noted in this work.

If the collector has a good eye for color tints, he will find that the color of the pycnia in fresh specimens is a good reliable distinguishing character always present. The color is quite constant with mature or nearly mature specimens. The pycnia always appear one to two months before the aecia. They are lighter in color tints when young and immature, but at the time the aecia appear they have assumed their final colors, which are the darker ones in the key.

The grouping and arrangement of both the pycnia and the aecia is also a good diagnostic character. Size and shape, though somewhat variable in some species, can well be taken into account. This key is primarily for use with fresh specimens, but will also aid much in naming dry material.

In collecting specimens, the proximity of any alternate host which might have borne the telia which infected the pines should be noted. Such information will supplement the key in making a determination. The key follows:

- I. Dehiscence of the peridium irregularly apical and longitudinal.<sup>2</sup>
  - A. Aecia usually less in height than in length.
    - a. Aecia very conspicuous,<sup>3</sup> usually in single extended rows.

<sup>1</sup> The writer acknowledges the assistance of N. Rex Hunt in the earlier work leading to the arrangement of this key.

<sup>2</sup> The term "longitudinal" refers to the direction of the main axis of the leaf.

<sup>3</sup> In general, aecia are very conspicuous when 2 to 3 or more millimeters high

\*Aecia irregularly flattened rhomboidal.

Pycnia orange rufous,<sup>4</sup> auburn, or chestnut in color and on yellowed chlorotic areas of the needles.

1. *Peridermium carneum* Bosc., aecial stage (I) of *Coleosporium carneum* (Bosc.) Jacks., on species of *Vernonia*.

\*\*Aecia linguiform to irregularly rhomboidal.

Pycnia cadmium orange to antique brown, on chlorotic areas.

2. *Peridermium Elephantopodis* (Schw.) Hedgc. & Hahn, I of *Coleosporium Elephantopodis* (Schw.) Thüm., on species of *Elephantopus*.

b. Aecia moderately conspicuous and in more or less extended rows.

Pycnia olivaceous black to brownish black, on slightly chlorotic areas.....

3. *Peridermium Ipomoeae* (Schw.) Hedgc. & Hunt, I of *Coleosporium Ipomoeae* (Schw.) Burrill, on species of *Convolvulus*, *Calonyction*, *Ipomoea* and *Quamoclit*.

Pycnia buckthorn brown to Dresden brown, on yellowed areas.

4. *Peridermium Fischeri* Kleb.,<sup>5</sup> \* I of *Coleosporium Sonchi-arvensis* (Pers.) Lév., on species of *Sonchus*.

Pycnia tawny to russet, on chlorotic areas.

5. *Peridermium floridanum* Hedgc. & Hahn, of which the related *Coleosporium* is not established, but may be the form on *Chrysopsis* in Florida.

Pycnia hazel to chestnut brown, on chlorotic areas.

6. *Peridermium Rostrupii* Ed. Fisch.,\* I of *Coleosporium Campanulae* (Pers.) Lév., on species of *Campanula* and *Specularia*.

c. Aecia small and inconspicuous, usually clustered or in short rows.

Pycnia dark olive to olivaceous black, on yellowed chlorotic areas.

7. *Peridermium fragile* Hedgc. & Hunt, I of *Coleosporium Laciniariae* Arth., on species of *Laciniaria*.

B. Aecia with height and length about equal.

a. Aecia and pycnia solitary or in extended rows.

Pycnia hazel to chestnut brown, on yellowed chlorotic areas.

and long, moderately conspicuous when 1 to 2 millimeters, and inconspicuous when less than 1 millimeter.

<sup>4</sup> Colors used are those of living specimens, unless otherwise noted, and are those of R. Ridgway, Color standards and color nomenclature, Washington, D. C., 1912.

<sup>5</sup> The specimens examined of species designated by an asterisk (\*) were dried exsiccati and the colors may not be quite comparable to those of living specimens.

8. *Peridermium ribicola* (Cooke & Ellis) Long, I of *Coleosporium ribicola* (Cooke & Ellis) Arth., on species of *Grossularia* and *Ribes*.
- b. Aecia and pycnia usually in short clustered rows.  
Pycnia deep chrome to raw umber, on slightly chlorotic areas.  
9. *Peridermium Helianthi* (Schw.) Hedgc. & Hunt, I of *Coleosporium Helianthi* (Schw.) Arth., on species of *Helianthus*.  
Pycnia olivaceous black to brownish black, on yellowed chlorotic areas..... 10. *Peridermium oblongisporium* Fuckel,<sup>6</sup> I of *Coleosporium Senecionis* (Schum.) Fries, on species of *Senecio*.
- C. Aecia usually greater in height than in length.  
a. Aecia and pycnia in single, sometimes extended, rows.  
Pycnia yellow ochre to Dresden brown, on slightly chlorotic areas.  
11. *Peridermium inconspicuum* Long, I of *Coleosporium inconspicuum* (Long) Hedgc. & Long, on species of *Coreopsis*.  
Pycnia old gold to buffy citrine, on yellowed chlorotic areas.  
12. *Peridermium californicum* Arth. & Kern, I of *Coleosporium Madiae* Cooke, on species of *Madia* and *Zonanthemis*.
- b. Aecia and pycnia clustered and in short rows.  
Pycnia grenadine red to mahogany red, on slightly reddened chlorotic areas..... 13. *Peridermium acicolum* Underw. & Earle,<sup>6</sup> I of *Coleosporium Solidaginis* (Schw.) Thüm., on species of *Aster* and *Solidago*.  
Pycnia orange rufous to mummy brown, on yellowed chlorotic areas.  
14. *Peridermium Terebinthinaceae* (Schw.) Hedgc. & Hunt, I of *Coleosporium Terebinthinaceae* (Schw.) Arth., on species of *Silphium* and *Parthenium*.
- II. Dehiscence of the peridium circumscissile.  
A. Aecia usually less in height than in length.  
a. Aecia large and conspicuous.  
Pycnia hazel to chestnut brown, on yellowed chlorotic areas.  
15. *Peridermium apocynaceum* (Cooke) Hedgc. & Hunt, I of *Coleosporium apocynaceum* Cooke, on species of *Ansonia*.
- b. Aecia small and inconspicuous.  
Pycnia orange chrome to English red, on reddened chlorotic areas.  
16. *Peridermium delicatulum* Arth. & Kern, I of *Coleosporium delicatulum*

<sup>6</sup> *Peridermium montanum* Arth. & Kern which may be distinct is included here.

(Arth. & Kern) Hedgc. & Long, on species of *Euthamia*.

B. *Aecia* about equal in height and in length.

*Aecia* small and inconspicuous.

*Pycnia* tawny to buckthorn brown, on slightly chlorotic areas.

17. *Peridermium minutum* Hedgc. & Hunt, I of *Coleosporium minutum*

Hedgc. & Hunt, on species of *Adelia*.

18. *Peridermium Weirii* Arth.<sup>7</sup>

#### A LIST OF NATURAL PINE HOSTS FOR THE PRECEDING SPECIES

*Pinus apacheca* Lemm. [*P. Mayriana* (Ellis) Sudw.], (13),<sup>8</sup> (16); *P. Banksiana* Lamb., (4), (6), (9), (13); *P. caribaea* More. [*P. heterophylla* (Ellis) Sudw. and *P. Elliotti* Engelm.], (1), (2), (3), (15), (16); *P. chihuahuana* Engelm., (3); *P. clausa* (Engelm.) Sarg., (1); *P. contorta* Loud. (*P. Murrayana* "Oreg. Com."), (1), (13), (18); *P. echinata* Mill., (1), (2), (3), (9), (11), (13), (14), (16); *P. edulis* Engelm., (8); *P. glabra* Walt., (1), (17); *P. Jeffreyi* "Oreg. Com.," (12); *P. nigra Poiretiana* Schneid. (*P. Laricio* Poir.), (1), (13), (16); *P. nigra austriaca* Schneid. (*P. Laricio austriaca* Endl.), (1), (13); *P. palustris* Mill., (1), (2), (3), (5), (7), (11), (14), (15), (16); *P. ponderosa* Laws., (1), (13); *P. ponderosa scopulorum* Engelm., (1), (13), (16); *P. pungens* Michx., (13), (16); *P. radiata* Don. (*P. insignis* Dougl.), (12); *P. resinosa* Ait., (13), (15), (16); *P. rigida* Mill., (1), (2), (3), (6), (7), (13), (14), (16); *P. serotina* Michx., (1), (2), (3), (13), (14), (16); *P. sylvestris* Linn., (1), (4), (10), (13); *P. taeda* Linn., (1), (2), (3), (7), (13), (14), (15), (16), (17); *P. Thunbergii* Parl., (13); *P. virginiana* Mill., (9), (11), (14).

#### AN ADDITIONAL LIST OF PINE HOSTS ARTIFICIALLY INFECTED

*P. apacheca*, (2); *P. canariensis* C. Smith, (2); *P. caribaea*, (13); *P. contorta*, (1), (2), (16); *P. Coulteri* Lamb., (1), (2), (13), (16); *P. glabra*, (16); *P. palustris*, (13); *P. pinea*, (8); *P. radiata*, (2), (13); *P. Sabiniana*, (1).

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<sup>7</sup> *Peridermium Weirii* Arth. is not known to the writer sufficiently to be incorporated in this key.

<sup>8</sup> Numbers in parenthesis refer to the numbers of the rust species in the foregoing key.

# CONTRIBUTIONS TO OUR KNOWLEDGE OF WESTERN MONTANA FUNGI—I MYXOMYCETES

PAUL W. GRAFF

The paucity of our literature on the distribution and especially the phenology of the Myxomycetes is decidedly noticeable. This is particularly true of our northwestern and southwestern states. Colorado has been more fortunate. When once their acquaintance has been made, these little forms of plant life have much to offer that is both interesting and attractive. The secrets of their hidden youth should be exposed, as well as their period of maturity, by greater publicity.

The following species of slime-moulds were collected by the writer in several localities of Montana west of the continental divide. While the list of species is not large, it is the first publication on these plants from that locality. In fact only a very few citations of species of Myxomycetes from this state are to be found in the mycological literature. The majority of the forms enumerated were collected in two quite separated regions. Many were gathered in the vicinity of Yellow Bay located half way up the eastern shore of Flathead Lake, about twenty miles from the city of Polson, and at the base of the Mission Mountains. A considerable portion of the remainder were found in the region around Missoula.

Because of seasonal and climatic conditions, the time best suited to the collection of Myxomycetes in this region is necessarily limited. This is true except for such very localized areas as are found to support a growth of *Thuja plicata*, the western arbor vitae, or other indicators of more or less continuous moisture. Until well into the spring the temperature is too cool. During the very dry summer season the conditions are such that they will cease to develop. The period of spring moisture which lasts usually from the middle of May to the same time in June is best suited to their growth. Again in the early autumn, if

precipitation occurs early enough, there will be a second season when they may develop. These two periods likewise define, though to a somewhat less extent, the time of appearance for the fleshy fungi.

The notes which accompany many of the species are not intended to be more than a statement of conditions as found in these collections. They are for the purpose of either amplifying the available descriptions, or of emphasizing certain points where authorities differ.

1. ARCYRIA DENUDATA (L.) Wetts. Verh. Zool.-Bot. Ges. Wien, 585. 1885-6.

*Clathrus denudatus* L. Syst. Nat. 1179. 1753.

On twigs of *Alnus tenuifolia* Nutt., Yellow Bay Creek, University of Montana Biological Station grounds, Flathead Lake, Lake County, July 9, 1918; Little Park Creek, Sapphire Mountains, Missoula County, May 27, 1924, at about 4,600 feet elevation.

This conspicuous little species is of very wide distribution. The attachment of its capillitium to the shallow cup, which remains after the rupture of the peridium, helps materially as a distinguishing mark. It is the only brilliantly colored form which has this characteristic, with the exception of the rose-colored *A. insignis* Kalchbr. and Cooke, for which the only North American locality seems to be the state of Massachusetts. The brightly colored red or reddish brown sporangia are from 2-3 mm. in height, and very conspicuous.

2. BADHAMIA NITENS Berk. Trans. Linn. Soc. 21: 153. 1852.

On decaying bark of *Betula fontinalis* Sarg., Little Dog Creek, vicinity of Olney, Flathead County, July 16, 1924; on twigs of *Acer glabrum* Torr., Miller Creek Canyon, Sapphire Range, Missoula County, May 20, 1925, at about 4,000 feet elevation.

Spores warted on the outside of the spore cluster, more faintly so on the side of contact, 11-13  $\mu$  in diameter with the usual size slightly less than the average, about 11.5  $\mu$ . From observations on number and arrangement of spores in relation to sporangial size, smoothness or tendency toward echinulation on the adjacent spore surfaces seems to be a matter of duration of close



contact and pressure during development. As a consequence this is a variable characteristic, and a failure as a diagnostic feature.

3. *BADHAMIA POPULINA* List. Jour. Bot. 13: 129. 1904.

On log of *Populus trichocarpa* T. & G., Yellow Bay, Flathead Lake, June 28, 1918.

Sporangia crowded, and most frequently sessile except for those outside the cluster, or in rare scattered cases, when they may possess a very short stalk. The spore clusters are not so variable in size as in the case of *B. capsulifera* (Bull.) Berk., with which this species is sometimes merged.

4. *BADHAMIA UTRICULARIS* (Bull.) Berk. Trans. Linn. Soc. 21: 153. 1852.

*Sphaerocarpus utricularis* Bull. Hist. Champ. Fr. 128, t. 417, fig. 1. 1791.

On log of *Larix occidentalis* Nutt., Blue Bay, Flathead Lake, July 12, 1918; on decaying log, Pattee Canyon, vicinity of Missoula, Missoula County, May 26, 1919.

5. *COMATRICHA FLACCIDA* (List.) Morg. Jour. Cinc. Soc. Nat. Hist. 16: 135. 1894.

*Stemonitis splendens* Rost. var. *flaccida* List. Monogr. Mycet. 112. 1894.

On twigs among moist leaves, South Fork of the Lolo, Bitter Root Mountains, Missoula County, September 18, 1925, at about 4,000 feet elevation, in stand of *Thuja plicata* D. Don.

Spores of this collection vary from 8.5–11  $\mu$ , a slightly higher size range than called for in the original description. The more usual size, however, falls within the characteristic dimensions, being about 9  $\mu$ .

6. *COMATRICHA TYPHOIDES* (Bull.) Rost. Vers. Syst. Mycet. 7. 1873.

*Trichia typhoides* Bull. Hist. Champ. Fr. 119, t. 477, II. 1791.

On decaying wood, Yellow Bay Creek, between Yellow Bay and Bear Trap Mountains, Mission Range, Lake County, July 2, 1921, at about 5,000 feet elevation.

The material though not plentiful is typical. This is not *C. typhoides* (Rost.) List., which Macbride identifies with *Stemonitis virginiensis* Rex. The error seems to be one of interpretation on the part of Lister.

7. CRATERIUM MINUTUM (Leers) Fr. Syst. Myc. 3: 151. 1829.  
*Peziza minuta* Leers, Fl. Herborn, 277. 1775.

On leaves, vicinity of Blue Bay, Flathead Lake, July 12, 1918; on decaying leaves, Rattlesnake Valley, Missoula County, May 18, 1920, at about 4,200 feet elevation; on leaves of *Acer glabrum* Torr., Yellow Bay Mountain, Mission Range, July 2, 1921, at about 4,000 feet elevation.

The cyathiform, nut-brown or greyish brown sporangia are very typical of the species. The plants in an early stage of development showed their characteristic rich yellow plasmodium.

8. CRIBARIA PURPUREA Schrad. Nov. Gen. Pl. 8. 1797.

On twigs of *Alnus tenuifolia* Nutt., under dense shade on the bank of Pattee Creek, vicinity of Missoula, September 27, 1925, at about 3,800 feet elevation.

9. CRIBARIA RUFA (Roth) Rost. Sluzowce Monogr. 232. 1875.  
*Stemonitis rufa* Roth, Fl. Germ. 1: 548. 1788.

On twigs, north slope of Mount Sentinel, Hell Gate Canyon, vicinity of Missoula, May 28, 1917, at about 3,600 feet elevation; on partially decorticated log of *Larix occidentalis* Nutt., Yellow Bay Mountain, Mission Range, July 15, 1921, at about 4,500 feet elevation.

10. DIDERMA RADIATUM (L.) Morg. Jour. Cinc. Soc. Nat. Hist. 16: 151. 1894.

*Lycoperdon radiatum* L. Sp. Pl. 2: 1654. 1753.

On partially decorticated log of *Pseudotsuga mucronata* (Raf.) Sudw., vicinity of Yellow Bay, Flathead Lake, July 13, 1920, at about 3,400 feet elevation; on decaying log, Yellow Bay Mountain, Mission Range, July 15, 1921, at about 5,000 feet elevation.

The material of the first collection is typical of the species, while that of the second approaches somewhat var. *umbilicatum* Meyl., with its drab-colored sporangia and irregular mode of

dehiscence. As in other American material, the spore size is slightly smaller than that denoted for the European, their range being 8–11  $\mu$  instead of 9–12  $\mu$ .

11. *DIDYMIUM ANOMALUM* Sturgis, Colo. Coll. Publ. Sci. Ser. 12: 444, pl. 2, fig. 6–8. 1913.

On bark of *Populus trichocarpa* T. & G., Yellow Bay, Flathead Lake, July 9, 1921.

The spores of this collection appear minutely warted rather than spinulose as suggested for the species by Macbride. The sporangia are of the plasmodiocarpous form, several centimeters long, and of a dull yellowish grey color. This seems to be a somewhat rare species, as it has been reported from but a few localities in either this country or Europe.

12. *DIDYMIUM QUITENSE* (Pat.) Torr. Flor. Myxom. 150. 1909.  
*Chondrioderma quitense* Pat. Bull. Soc. Myc. Fr. 11: 212. 1895.

On fallen leaves of *Betula fontinalis* Sarg., Little Dog Creek, vicinity of Olney, Flathead County, July 14, 1924, at about 3,500 feet elevation; on fallen leaves, South Fork of the Lolo, Bitter Root Mountains, May 23, 1925.

The material of these collections is very close in its characteristics to *Didymium difforme* (Pers.) Duby., which has been reported as rare within the United States. The spore wall is rough, but the reticulations, as well as the violaceous tinge of the spore coat, are decidedly obscure. Both must be very closely related, and it would seem somewhat better to give this varietal distinction under the older name of *D. difforme*.

13. *FULIGO INTERMEDIA* Macb. N. Am. Slime-Moulds, Ed. II, 30. 1922.

On moss plants under dense shade on the stream bank, Pattee Canyon, vicinity of Missoula, July 12, 1925, at about 4,000 feet elevation.

As this has only been reported from the Rocky Mountain region (the original collections were made in Colorado from whence it seems only to have been reported previously) this may

be but a mountain variety. The genus is composed of such similar and overlapping forms that they offer considerable difficulty in the establishment of distinctive species on sufficient basis for clarity of interpretation. Lister places this as var. *excorticata* under *F. cinerea* Morgan, but it has a cortex!, though thin and fragile.

14. HEMITRICHIA VESPARIUM (Batsch) Macb. N. Am. Slime-Moulds, 203. 1899.

*Lycoperdon vesparium* Batsch, Elench. Fung. Suppl. I, 253-256, fig. 171a-c, 172a-d. 1786.

On twigs of *Betula fontinalis* Sarg., Miller Creek Canyon, Sapphire Range, Missoula County, April 27, 1920, at about 4,500 feet elevation.

This brilliantly colored species is easily recognized by its bright red color and clustered habit in which the sporangia are densely crowded. The spores are not smooth, as described by Rostafinski, but clearly though not strongly warted.

15. LAMPRODERMA COLUMBINUM (Pers.) Rost. Vers. Syst. Mycet. 7. 1873.

*Physarum columbinum* Pers. Obs. Myc. 1: 5. 1796.

On twigs of *Betula* sp., Yellow Bay, Flathead Lake, July 9, 1918; on twigs of *Thuja plicata* D. Don, South Fork of the Lolo, Bitter Root Mountains, September 18, 1925.

The material was in fine condition, and typical in all respects.

16. LAMPRODERMA SAUTERI Rost. var. ROBUSTUM (Ellis & Ev.) comb. nov.

*Lamproderma robustum* Ellis & Ev. in Mass. Monogr. Myx. 99. 1892.

On partially decorticated log of *Pinus ponderosa* Dougl., Yellow Bay, Flathead Lake, July 3, 1921.

Sporangia globose to subglobose, 1-1.5 mm. in diameter, purplish black to black, from a black stalk which is short or as long as the sporangial diameter; columella short, thick, nodulose or widened and flat at the top from which the dense capillitium grows; capillitium dark to purplish brown, delicate and much

branched, anastomosing near the outer periphery to form a fine-meshed network; spores dark purple-brown, minutely echinulate, 12–15  $\mu$ .

This American variety is very close to *L. Sauteri* of Rostafinski, but cannot claim relationship with *L. violaceum* Rost., as Lister would have it. Macbride, in his first edition, considers it identical with *L. Sauteri*, but reconsiders and makes them distinct species in the second edition of his monograph. Neither extreme seems to suit the situation. The American material is too close to *L. Sauteri* to be considered separate from it, but distinct enough for varietal status.

17. LEPIDODERMA TIGRINUM (Schrad.) Rost. Vers. Syst. Mycet.  
13. 1873.

*Didymium tigrinum* Schrad. Nov. Gen. Plant. 22. 1797.

On twigs among moist leaves, Deer Creek, vicinity of Bonner, Missoula County, May 21, 1922, at about 4,300 feet elevation; on twigs of *Alnus* sp., Blackfoot Valley, near Twin Creeks, May 30, 1923, at about 3,800 feet elevation.

The purplish, scaly sporangia with dark brown stipes were quite characteristic. These grew from a pale yellow to orange, or in age brownish, hypothallus. The plasmodium was of a pale yellow color.

18. LYCOGALA EPIDENDRUM (Buxb.) Fr. Syst. Myc. 3: 80. 1829.

*Lycoperdon epidendrum* Buxb. En. Pl. Hal. 203. 1721.

On decaying log, Yellow Bay Mountain, Mission Range, July 12, 1918, at about 4,200 feet elevation; on decorticated log of *Pinus ponderosa* Dougl., Yellow Bay, Flathead Lake, July 9, 1921; on decaying sticks, Belmont Creek, Blackfoot Valley, Missoula County, May 20, 1925, at about 3,800 feet elevation; on partially decorticated log of *Pseudotsuga mucronata* (Raf.) Sudw., Deer Creek, vicinity of Bonner, Missoula County, July 12, 1925, at about 4,000 feet elevation.

19. PHYSARUM AURISCALPIUM Cooke, Ann. Lyc. Nat. Hist. N. Y.  
11: 384. 1877.

On twigs of *Alnus tenuifolia* Nutt., South Fork of the Lolo, Bitter Root Mountains, May 23, 1925, at about 4,200 feet elevation.

This is possibly synonymous with *Physarum oblatum* Machb., as it is considered by Lister. The two are certainly very closely related, if not identical. Further study and careful comparisons are needed before a positive conclusion can be reached. The larger lime knots of the capillitium and their color, which tends toward the orange-yellow as described by Cooke, seem to be the main point of separation.

20. *PHYSARUM BITECTUM* List. Monogr. Mycet. Ed. II, 78. 1911.

*Physarum Diderma* List. in Jour. Bot. 29: 260. 1891, non Rost.

On sticks of *Betula* sp., Yellow Bay, Flathead Lake, July 2, 1921, at about 3,000 feet elevation; on coniferous twigs, Deer Creek, vicinity of Bonner, May 21, 1922, at about 3,800 feet elevation.

There seems to be some difficulty in distinguishing this species from *Physarum sinuosum* (Bull.) Weinm., with which it appears to be related. The distinction is based largely on spore character, these being decidedly spinulose rather than smooth, and on the purplish color of the inner sporangial wall. In these collections calcium salts are present in the capillitium in the form of coarse irregular white nodules, having very short connections.

21. *PHYSARUM BRUNNEOLUM* (Phill.) Mass. Monogr. Myx. 280, fig. 221-222. 1892.

*Diderma brunneolum* Phillips, Grevillea 5: 114. 1877.

On twigs of *Thuja plicata* D. Don, South Fork of the Lolo, Bitter Root Mountains, September 18, 1925, at about 4,500 feet elevation.

Sporangia densely gregarious, stalked, 5-7.5 mm. in diameter, slightly smaller than the typical size for the species. The spores, on the other hand, are of the usual size, 8-10  $\mu$  in diameter, and spinulose.

22. *PHYSARUM CARNEUM* G. List. & Sturgis, Jour. Bot. 48: 73. 1910.

On decaying log of *Larix occidentalis* Nutt., Little Dog Creek, vicinity of Olney, Flathead County, July 16, 1924, at about 3,700

feet elevation; on twigs of *Acer glabrum* Torr., Rattlesnake Valley, Missoula County, May 24, 1925, at about 4,200 feet elevation.

Sporangia developing from a mustard-yellow plasmodium, usually gregarious but rarely scattered, globose or subglobose, short-stipitate, ochraceous yellow, or with the lower portion varying from flesh-colored to reddish, 0.5–0.6 mm. in diameter. Stalk flesh-colored or reddish, 0.2–0.3 mm. in length. Spores 8–9  $\mu$  in diameter, the majority approaching 9  $\mu$ , slightly larger than those of Sturgis' material. This species differs from *P. citrinellum* Peck, with which it seems related, in having smaller spores, and in the fact that the latter develops from a greenish white plasmodium with its sporangia borne upon orange-red stalks.

23. *PHYSARUM DIDERMA* Rost. Sluzowce Monogr. 110. 1875.

*Physarum testaceum* Sturgis, Colo. Coll. Publ. Sci. Ser. 12: 18. 1907.

On twigs, Yellow Bay Creek, between Bear Trap and Yellow Bay Mountains, Mission Range, July 12, 1919.

The material of this seldom reported species is very typical, and it is a pleasure to add another locality to the few already enumerated.

24. *PHYSARUM POLYCEPHALUM* Schw. Syn. Fung. Car. 63. 1822.

On decaying log of *Betula fontinalis* Sarg., Yellow Bay, Flathead Lake, July 10, 1920; on leaves and twigs of *Populus tremuloides* Michx., Pattee Canyon, vicinity of Missoula, June 6, 1923, at about 3,800 feet elevation.

Spores 8–10  $\mu$  in diameter, comparing more closely with the dimensions of European material; Macbride reports 9–11  $\mu$  as usual for North American collections. The species is common, widely distributed, and quite variable in appearance, particularly respecting sporangial form.

25. *PHYSARUM SINUOSUM* (Bull.) Weinm. in Fr. Syst. Myc. 3: 145. 1829.

*Reticularia sinuosa* Bull. Hist. Champ. Fr. 94, t. 446, fig. 3. 1791.

On leaves, Yellow Bay, Flathead Lake, Lake County, July 17, 1921; on leaves, Deer Creek, vicinity of Bonner, Missoula County, August 15, 1925, at about 3,600 feet elevation.

In both cases the sporangia developed the plasmodiocarpous type, growing over the leaves in the characteristic sinuous and branching manner. The prominent snowy white calcareous deposit is well developed, though reported as sometimes wanting, or at least reduced in quantity, in our American forms. The well-developed capillitium does not have as coarse nodules as appear in the case of *P. bitectum* List., with which this species is related, though the general appearance is much the same. Spores smooth, 8–10  $\mu$  in diameter.

26. *PHYSARUM TENERUM* Rex. Proc. Phil. Acad. Sci. 1890: 192. 1891.

On partially decorticated log of *Betula papyrifera* Marsh., shore of Yellow Bay, Flathead Lake, July 12, 1919.

So far as I have been able to ascertain, this is the first report of this species from any of the northwestern states. The growth was quite luxuriant for this delicate little species. Sporangia globose, erect or nodding, with a total height of from 1–2.5 mm., the sporangial head 0.4–0.6 mm. in diameter, the stalk slender, 0.5–1.8 mm. in length. The plasmodium was yellowish in color.

27. *RETICULARIA LYCOPERDON* Bull. Hist. Champ. Fr. 95, t. 446, fig. 4. 1791.

Growing from check cracks on stump of *Pseudotsuga mucronata* (Raf.) Sudw., Yellow Bay, Flathead Lake, June 27, 1918; on stool of *Agropyron spicatum* Scribn. and Sm., base of Mount Sentinel, vicinity of Missoula, May 11, 1924; on grass in street parking, Missoula, April 17, 1925.

This species appears quite frequently in western Montana. One is likely to find it at any time, after the warm spring rains, till the dryer summer season begins, and on a variety of substrata. In the second and third collections the plasmodium had completely left the ground, and the large aethalium was entirely supported in a horizontal position by the grass stalks.



28. STEMONITIS FLAVOGENITA Jahn, Abh. Bot. Ver. Brand. 45: 265. 1904.

On fallen trunk of *Betula* sp., on north slope of Yellow Bay Mountain, Mission Range, July 12, 1919, at about 5,200 feet elevation.

Plants growing from the characteristic yellow plasmodium, and having the other distinctions which differentiate this species from *S. ferruginea* Ehr., with which it was confused by the earlier workers.

29. STEMONITIS FUSCA Roth, Mag. Bot. 2: 26. 1787.

On bark of a standing but partially decorticated *Pseudotsuga mucronata* (Raf.) Sudw., Yellow Bay, Flathead Lake, June 28, 1916.

The specimens have the superficial appearance of var. *rufescens* Lister, but the spore size is somewhat larger, 7-9  $\mu$ , approaching more nearly the average for the species. Spore reticulations are so slightly developed as to be scarcely discernible.

30. STEMONITIS SPLENDENS Rost. Sluzowce Monogr. 195. 1875.

On the bark of a partially decorticated log of *Populus trichocarpa* T. & G., Yellow Bay, Flathead Lake, July 6, 1918.

Though sometimes confused with *S. fusca* Roth, this species is readily distinguished by its capillitium which arises from the columella at more distant intervals. It is much more open and coarse throughout than will be found in the case of *S. fusca*. The hypothallus is purplish rather than brown.

31. STEMONITIS UVIFERA Macbr. N. Am. Slime-Moulds, Ed. II, 161, pl. 20, fig. 8, 8a-8c. 1922.

On fallen trunk of *Picea Engelmanni* (Parry) Engelm., South Fork of the Lolo, Bitter Root Mountains, Missoula County, May 23, 1925, at about 4,300 feet elevation.

The spores of this species have been described as "marked with a cap of minute spines on the side facing outward in the cluster." This seems to be a variable characteristic, and governed by the closeness of contact. When the spore clusters develop somewhat loosely, the amount of surface upon which spines appear increases.

They may even develop over the entire surface. These "spore clusters" should by no means be confused with the spore-balls of the Ustilaginaceae or other similar structures for they are merely the chance contact of a varying number of independent spores. The condition in this case is similar to that noted above for *Badhamia nitens* Berk., where the amount of echinulation appearing on the spore surface varies according to the internal arrangement and pressure during development.

32. *TRICHIA AFFINIS* De Bary, in Fuckel. Sym. Myc. 336. 1869.

On decaying wood of *Larix occidentalis* Nutt., Yellow Bay, Flathead Lake, July 17, 1918; on wood of *Pseudotsuga mucronata* (Raf.) Sudw., north slope of Yellow Bay Mountain, Mission Range, July 12, 1919, at about 5,000 feet elevation.

Sporangia of a shining golden yellow, changing with age to ochraceous. The luster remains prominent through both the period of development and maturity. Macbride considers this synonymous with *T. persimilis* Karst.

33. *TRICHIA DECIPIENS* (Pers.) Macbr. N. Am. Slime-Moulds, 218. 1899.

*Arcyria decipiens* Pers. in Ust. Ann. Bot. 15: 35. 1795.

On partially decorticated log of *Populus trichocarpa* T. & G., Yellow Bay, Flathead Lake, July 6, 1921.

Very similar in size and general appearance to *Hemitrichia clavata* (Pers.) Rost., which is yellowish to olivaceous in color, rather than the olive to olivaceous brown of this species.

34. *TRICHIA PERSIMILIS* Karst. in Not. Faun. Flor. Fenn. 9: 353. 1868.

On twigs of *Acer glabrum* Torr., Deer Creek, vicinity of Bonner, May 21, 1922, at about 3,800 feet elevation.

Closely related to *Trichia scabra* Rost., and *T. affinis* De Bary. It differs from the former in not being as brilliantly colored, and in having slightly smaller peridia, but larger spores. From *T. affinis* it also differs in its more somber color, smaller peridia, and also smaller spores; *T. affinis* having larger spores than either of the other species. The spore sizes for the three, as represented in

these collections, are *T. scabra* 10–12  $\mu$ , *T. persimilis* 12–14  $\mu$ , and *T. affinis* 13–15  $\mu$ ; comparable to the dimensions given by Lister (Monogr. Mycet. Ed. III, 1925). With this Macbride does not agree, but gives the spore size in the several instances as the same, 10–12  $\mu$ .

35. *TRICHIA SCABRA* Rost. Sluzowce Monogr. 258. 1875.

On twigs, Yellow Bay Creek, between Bear Trap and Yellow Bay Mountains, Mission Range, July 2, 1921, at about 4,600 feet elevation.

This is a species which seems, from the available information, more common to our northwestern region than to the eastern states.

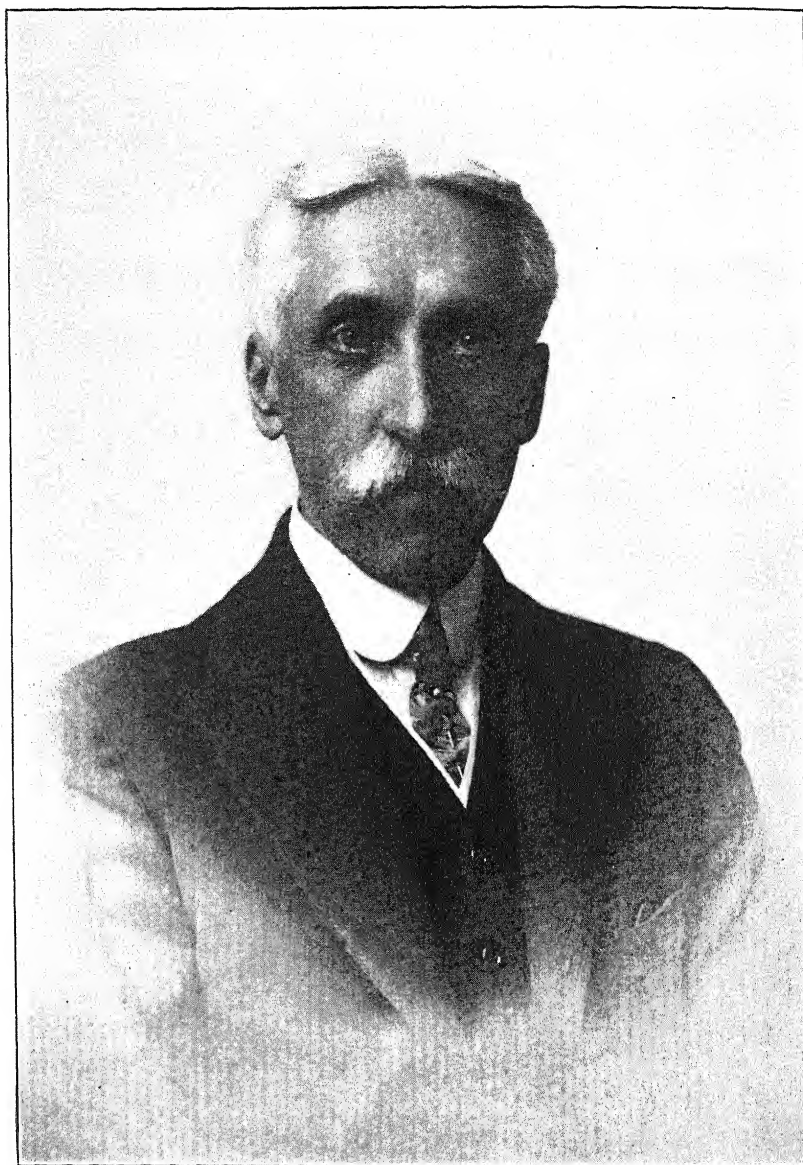
36. *TRICHIA VARIA* (Pers.) Fr. Syst. Myc. 3: 188. 1829.

*Stemonitis varia* Pers. in Gmel. Syst. Nat. 2: 1470. 1791.

On twigs of *Acer glabrum* Torr., Yellow Bay, Flathead Lake, July 12, 1919.

Spores minutely warted, 13–16  $\mu$  in diameter, yellow in color.

COLUMBIA UNIVERSITY,  
NEW YORK CITY



JOSEPH CHARLES ARTHUR

# MYCOLOGIA

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## DOCTOR ARTHUR'S RUST WORK

FRED J. SEAVER

(WITH PLATE 14)

Dr. J. C. Arthur of Purdue University has just completed his monumental and long-looked-for work on the Uredinales in the North American Flora (except indices), the first part having been issued March 6, 1907, and the last part October 26, 1927. This entire work comprises 766 pages of text which has required twenty years for its publication, and half as much longer of continuous effort to compile. Probably no other piece of mycological work in America has been carried out so persistently, and, while some may differ as to the method of presentation, all mycologists will agree that he has brought together under one cover a vast amount of knowledge in this particular group which will be invaluable to coming generations, whether they may choose to follow his classification or not.

The work is based largely on his own collection of Uredinales housed in Purdue University, Agricultural Experiment Station, representing over 50,000 collections of rusts obtained from all parts of the world but chiefly from North America. This is probably the largest rust collection in America. Much time has also been spent on the rust collection of The New York Botanical Garden which has been arranged by him and his assistants to follow the treatment in the North American Flora. It represents the second largest collection of rusts in the country, with nearly 40,000 collections. He has not, however, confined his attention to these two collections but has studied in other

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large herbaria at home and abroad. He has made a number of trips to Europe in order to consult with Uredinologists of other countries.

Dr. Arthur is one of the oldest of American mycologists and an outstanding figure in that field, having more than two hundred and fifty titles to his credit. It has always been a satisfaction to collectors in out-of-the-way places to know that at least, their rust collections could be thoroughly worked over and the results submitted within a reasonable time. This is probably not true of any other large group of fungi. In this way the rusts of various islands of the West Indies and the countries of Central America have been recorded by him, although he himself has never visited any of them.

The writer's first intimate knowledge of Dr. Arthur's work was gained during the spring of 1903 while a student in the State University of Iowa, having been delegated by Professor T. H. Macbride to assist Dr. Arthur in his rust work for that season, at Purdue University. The ten weeks spent in Indiana were both profitable and interesting. Although not particularly interested in the rusts, I was much impressed by the methodical manner in which the culture work was conducted and the great care in handling all details connected with his rust herbarium. Since coming to The New York Botanical Garden in 1908 we have carried on an almost continuous correspondence and exchange of materials and to him I am indebted for the determination and publication of my Trinidad rusts under the title, "Uredinales Collected by Fred J. Seaver in Trinidad." Among the new species reported in this paper I am naturally proud of the one dedicated to me (*Puccinia Seaveriana*), a minute species occurring on the leaves of a composite tree.

Many other young mycologists have worked with him for longer or shorter periods and I am sure all of these and many others who have not been so intimately associated with him will join in commending Dr. Arthur for his present accomplishments as well as in wishing him still many years of active work in the field of research. We take pleasure in presenting at this time a photograph taken on his seventy-third birthday.

THE NEW YORK BOTANICAL GARDEN,  
BRONX, NEW YORK CITY.

## TRILLIUM RUST <sup>1</sup>

M. F. BARRUS

(WITH PLATES 15 AND 16)

An *Aecidium* on *Trillium recurvatum* was collected by A. B. Seymour in 1882 at Pine Hills, Union County, Illinois. Two years later this was described by Burrill (1) as *Aecidium Trillii* n. sp. No printed record of this *Trillium* rust has since appeared from Illinois and many years passed without any further reference to it anywhere. In 1907 it was found on *Trillium grandiflorum* at Taughannock Falls near Ithaca, N. Y., by H. H. Whetzel, M. B. Thomas, and the writer. Since that time it has been observed there nearly every year on both *T. grandiflorum* and *T. erectum*. House (2) reports finding it in 1923 on *T. erectum* and *T. undulatum* near Newcomb, Essex County, N. Y. A collection of an *Aecidium* on a plant labeled "*Trillium obovatum* Ledeb.," probably *T. kamtschatikum* Pall., was made by W. L. Kamarov on July 6, 1908, in Kamchatka and determined by W. A. Tranzschel to be *A. Trillii* Burrill. This collection was deposited in the Institutum cryptogamicum Horti Botanici Petropolitani. A specimen from this collection is in the Office of Mycology and Disease Survey of the Bureau of Plant Industry, Department of Agriculture, Washington, D. C. An examination of this specimen by the writer shows the rust to be identical with *A. Trillii* found at Taughannock Falls.

The prevalence and distribution of this rust as it occurs at Taughannock Falls has been studied from year to year since 1915 primarily with the hope of discovering the alternate host. Some years it has not been seen at all. Most years only a few rusted plants could be found, sometimes only one or two being

<sup>1</sup> The writer gratefully acknowledges the advice given by Professor H. H. Whetzel and the use of his notes on *Trillium* rust; the helpful suggestions extended by Doctors H. S. Jackson, F. D. Kern, and J. C. Arthur; the assistance of Mr. J. G. Horsfall in making spore measurements and spore germination tests; and the assistance of others in the preparation of this paper. The photographs used were made by Mr. W. R. Fisher.

located and those but slightly rusted, although both *Trillium erectum* and *T. grandiflorum* grow there profusely. But during three years, at least,—1915, 1922, and 1925—it was abundant on both species of *Trillium* within the particular area where it occurs, and the affected plants had from one to several lesions.

At Taughannock Falls, *Aecidium Trillii* had been confined to a particular area on the north side of the gorge. This area is about half way up the gorge from the lake to the falls and is near the foot of the path that leads to the top on that side. The affected plants do not occur within this area in exactly the same location year after year. In 1915 and 1922, plants affected with the rust were found abundantly at the base and sides of the slope but the limits of the area were not determined. In 1920 and 1921 they were confined to a space about 5 rods in its widest diameter, located on the slope facing south. Only twelve rusted plants were found in 1923 and twelve in 1924, and these were located within the same area. Since 1924 the location of the rusted area has been near by but on the slope facing to the southeast. In 1925 affected plants were discovered along the side of this slope and at its foot for a distance of 25 rods in a southeast and northwest direction, the area being top-shaped with its widest diameter on the side of the slope at the northwest. During 1926 and 1927 the rusted plants have been found only on the slope at the northwest end of this area. This slope is very steep, nearly 45°, and trilliums grow sparingly there, although near the foot where the slope is more gradual, *Trillium erectum* is abundant. Both species are very numerous on the flat at the bottom of the slope and at various places throughout the entire length of the gorge which is nearly a mile long. Yet at no time, since these observations have been made, have rusted plants been found elsewhere in the gorge nor has the rusted area been greater than 15 × 25 rods.

The lesions first appear after the plants have passed the peak of blooming. They have been first observed at Taughannock Falls during the latter half of May or the first half of June, depending on the season. During 1922, pycnia and immature aecia were found on May 14, which is earlier than in any other year. Within a week after the appearance of the pycnia, the



aecia may be open but sometimes it may be two or three weeks before they reach maturity. A secondary, more or less complete, ring of aecia may surround those first formed (PLATE 15, A), or there may be an extension of the spot in which aecia are produced. A light yellow zone surrounds the primary ring before the secondary ring appears and a similar zone may later surround the latter (PLATE 15, B). New spots were found on young plants and on green parts of older ones at a time when older spots had become dry and dead and these, apparently, were due to more recent infections.

The spots, before the aecia are mature, are lemon-yellow in color and vary in size, some being as much as 2 cm. in diameter. The pycnia, which have a bright red color, are scattered over the central area of the spot, in some cases only on the lower and in others on both the lower and upper surface of the leaf. The aecia then are pearly-white, cone-shaped projections occurring in a zone about the pycnia. The leaf tissue of the affected area is somewhat thicker than healthy tissue but is not materially distorted.

The description of *Aecidium Trillii* given by Burrill (1) and by Arthur (3) agrees well with the specimens under observation. Fresh specimens have pycnia with a distinctly red color when young. These are  $83\text{--}100\ \mu$  wide and  $76\text{--}92\ \mu$  high. The ostiolar filaments are  $65\text{--}85\ \mu$  long and, near the apex, the agglutinated mass of filaments and pycniospores may become nearly as wide as the pycnium. The pycniospores are hyaline, ovoid to oblong bodies, about  $2 \times 4\ \mu$  in diameter. The aeciospores are  $12.6\text{--}23.4 \times 14.4\text{--}25.2\ \mu$ , mostly  $16.2\text{--}18 \times 18\ \mu$  in diameter (PLATE 15, C). Fair to good germination of aeciospores was obtained within 24 hours when they were placed in distilled water on glass slides and kept at  $15\text{--}20^\circ\text{C}$ .

The uredinal stage of a rust on *Brachyelytrum erectum* (Schreb.) Beauv. was observed within the *Trillium* rust area at Taughanock Falls on July 4, 1922. Because of its location and because no rust had been reported on this grass, it was thought that it might be the alternate host of *Aecidium Trillii*. The rust was abundant on the grass growing among the rusted trilliums but not elsewhere. Later in the season telia were present in the

rusted lesions. During 1923 and 1924 this rust was not found. These were years when rust on the trilliums was scarce. Only four rusted stools of this grass were found during 1925 but these were severely affected. They were growing on the slope facing southeast which was the location of rusted trilliums that year. During 1926 only three stools of rusted grass were found, these being the same plants that were affected the year before. The only rusted trilliums found during 1926 were in the vicinity of these rusted grass plants and on the slope below them. These same stools of grass, two young plants immediately adjoining, and two others, respectively 14 and 27 feet away, were the only ones affected by rust during 1927 and it was only in this vicinity that the *Trillium* rust occurred that year. A careful search of all trilliums and of *Brachyelytrum erectum* growing within the area at Taughannock where rusted trilliums had been found in past years, and in the surrounding area, was made during the summer and fall of 1927. The grass occurs mainly on the slope facing south, midway between the foot and head of the slope, and occupies an area of approximately  $5 \times 12$  rods. This area coincides in part with the *Trillium* rust area but extends somewhat farther west. The *Trillium* rust area some years extends somewhat farther east and southeast (down the slope) than the area occupied by the grass. The grass is not particularly abundant, even within the *Trillium* rust area, as only about 200 stools or growing plants can be found. A few plants have been found along the slope above, but none at all on the floor of the gorge, where trilliums grow profusely, nor on the other slopes. The limitation of the distribution of *Trillium* rust largely to the area in which the grass occurs, and of the grass rust in any year to the particular location of rusted trilliums, as well as the occurrence of the grass rust only in years when *Trillium* rust is abundant or present, seemed to indicate a relationship between these rusts.

#### INOCULATION EXPERIMENTS

In the fall of 1922, rusted leaves of *Brachyelytrum erectum* were placed in wire cages for wintering and one plant was transplanted near some red and white trilliums in the writer's garden

at a place partly shaded by trees. The following spring, the wintered leaves were suspended over trillium plants placed in pots under bell jars on a greenhouse bench. Rusted leaves were also placed over trilliums growing in the garden which were then covered with bell jars. In no case was any infection of the trilliums obtained from these inoculations. The temperature during the inoculation period was very high and it was quite dry out-of-doors, although the greenhouse plants were kept wet. During 1923, there was very little infection on the trilliums at Taughannock.

As no rusted grass material could be located during 1923 and 1924, further attempts to inoculate trilliums were necessarily postponed until 1926. The previous autumn, rusted grass leaves had been collected and placed in a wire cage. One clump of rusted grass had been planted in a pot and placed in the garden near red and white trilliums. On May 2, rusted grass leaves were placed beneath and above other young white trilliums in the garden and the plants given half shade with a slotted cage. Still other trilliums were also similarly treated but were shaded only by trees. On the same day rusted grass leaves were placed on the upper surface of three white trilliums at Taughannock. A six-hour rain occurred during the afternoon of the following day. On May 18, two rust spots showing pycnia were observed on one white trillium inoculated in the garden. One of these spots never developed mature aecia. The other inoculated plants in the garden did not become affected. On May 23, each of the three white trilliums inoculated at Taughannock showed two spots. Only ten other rusted trilliums could be found at Taughannock during 1926.

Two red trilliums from the garden were transplanted to pots and placed in a cool greenhouse on May 4. Old rusted grass leaves were placed about their base and suspended from the top of a bell jar that was placed over them. The grass leaves and plants were well moistened. Three days later the bell jar was removed. On May 12, the leaves were densely spotted with pycnia on upper and lower surfaces. Pycnia were present on the sepals and on the upper end of the flower stalk. Mature aecia were observed on May 26.

One red trillium was inoculated on May 13 by suspending over it the rusted grass leaves used in the inoculation of May 4 and was then watered and covered with bell jar for three days. On May 24, two spots containing pycnia had developed on the leaves and, on June 2, mature aecia were observed.

On April 28, 1927, two red trilliums and three white ones, placed in pots and transferred from the garden to cool greenhouse, were inoculated by suspending over them old rusted grass leaves collected the previous autumn. They were well wet down and left covered with bell jars for four days. Pycnia were observed on the red trilliums on May 9. On May 13, a count showed 155 pycnial spots on the leaves of one plant and 79 on those of the other. One of the white trilliums showed but one spot and the others none. This does not indicate that the white trilliums showed resistance as the material used for inoculation had molded considerably. All the lesions were small (3 mm. in diameter or less), some scarcely distinguishable to the eye. The aecia were beginning to open on May 23 and their development continued on leaf veins and at apex of the stalk until the last of July, the tissue of the leaf-blades being brown and dry for the most part at that time.

The results of inoculations on *Brachyelytrum erectum* with aeciospores from *Trillium* were not so successful. From 37 inoculations made in 15 series at 12 different times from 1925 to 1927, infection took place in only three cases. Observations were not made of the time when the first evidence of infection occurred. When, on May 24, 1925, three rusted white trilliums, obtained at Taughannock, were planted near grass which had been transplanted to the garden the year before, infection took place some time during the summer, for numerous telia were present on the leaves especially on the lower ones. No infection occurred on the grass, obtained at the same time as the other, and which was growing in another part of the garden. When, on June 11 of the same year, aecia on red trillium were rubbed against the wet leaves of the grass which was then placed under a bell jar at 15° C. for 24 hours and then in an inoculation chamber where the temperature varied from 18–24° C., several uredinia appeared on one leaf by July 26. None occurred on check

plants treated similarly except not inoculated. When grass in another pot was inoculated on June 15, 1926, with aeciospores from red and from white trilliums in the same manner and with the same treatment as with the inoculation of June 11, several uredinia were observed on one leaf on July 19. Only a few inoculations were made during 1926 and 1927, as inoculum was scarce, and none of them resulted in infections.

Inoculations of *Brachyelytrum erectum* with urediniospores were likewise not very successful. Of 5 inoculations made in 1925 and 1 in 1927, only two resulted in infections. One made on July 28, 1925, by brushing urediniospores, obtained from the grass inoculated with aeciospores on June 11, and then placed in inoculation chamber for 5 days, showed 32 sori on 17 leaves on October 2. Of these sori, 18 were telia. One plant inoculated August 3, 1925, in the same manner and having the same treatment as the other, showed 1 uredinium on September 11 but no others developed. Check plants did not develop any rust.

Undoubtedly many of the unsuccessful attempts at securing infection in the above-mentioned experiments were due to improper attention to details. Necessary and frequent absences of the writer from Ithaca also prevented many observations of the inoculated plants from being made.

#### OBSERVATIONS AND CONCLUSIONS

The rust on *Brachyelytrum erectum* usually occurs most abundantly on the leaves of the lower part of the plant, the upper two or three often being free from rust spots. The apical end of the leaf is, as a rule, most severely affected but spots may occur over any part of the upper surface (PLATE 16, A and B). The lower leaves become quite generally covered with sori which may even appear on the sheath.

On the upper surface the rusted areas are somewhat elongated, with a color varying from clay to dark olive buff. Dark spots within these areas indicate the location of sori beneath. When sori are numerous, the leaf tissue between also becomes discolored. By the time telia are produced, the tissue about them has died. The apical end of badly affected leaves may be brown and dead by late summer.

Urediniospores germinate readily in distilled water at 15° C. within 24 hours. Teliospores do not germinate readily. Although tests were made from October to May, March 30 was the earliest date at which germination took place. The most abundant germination was secured in May. A four-celled promycelium, about  $7 \times 60 \mu$ , having four sporidia, developed within 24 hours.

This rust resembles very closely the one described by Halsted (4) as *Uromyces digitatus* occurring on *Leersia virginica* Willd. and later named *Uromyces Halstedii* by De-Toni (5). The principal difference seems to be, according to H. S. Jackson,<sup>2</sup> that this *Uromyces* has 2-3 slightly sub-equatorial pores in the urediniospores (PLATE 16, C) as compared to the 3-4 equatorial pores in *Uromyces Halstedii* described by Arthur (6). The telia are slightly wider and the epidermal covering less persistent. Also the digitate projections of the teliospores (PLATE 16, D) are longer, 4-8  $\mu$ , than those of *Uromyces Halstedii*. These differences do not seem to be sufficient for the constitution of a new species. It seems more logical to consider the rust on *Brachyelytrum erectum* as *Uromyces Halstedii* De-Toni. Should inoculation of *Leersia* with the aeciospores from *Trillium* or with the urediniospores of *Uromyces Halstedii* give negative results, it might be held to be a physiological race. No aecial host for this rust has previously been recorded nor has it been reported earlier as occurring on any grass host other than *Leersia virginica* Willd. and *L. oryzoides* (L.) Sw. On the former it has been found in Alabama, Iowa, Louisiana, Maryland, Wisconsin; and on the latter in Delaware, South Dakota, Texas and Wisconsin. It has also been reported by Ito (8 and 9) from Japan on *Leersia oryzoides* (L.) Sw., var. *japonica* Hack.

The synonymy appears to be as follows:

*Aecidium Trillii* Burrill, Bot. Gaz. 9: 190. 1884.

*Uromyces digitatus* Halsted, Jour. Myc. 3: 138. 1887.  
Not *U. digitatus* Wint. 1886.

*Uromyces Halstedii* De-Toni, in Sacc. Syll. Fung. 7: 557.  
1888.

*Uromyces Halstedii* Ludw., Bot. Centr. 37: 120. 1889.

<sup>2</sup> Letter of Sept. 29, 1927.

- Caecomurus Halstedii* Kuntze, Rev. Gen. 3: 450. 1898.  
*Uromyces ovalis* Diet., Bot. Jahrb. 37: 97. 1905.  
*Nigredo digitata* Arth., Result. Sci. Congr. Bot. Vienna  
343. 1906.  
*Nigredo Halstedii* Arth., North Am. Fl. 7: 226. 1912.  
*Ontoteliium digitatum* Sydow, Ann. Myc. 19: 174. 1921.  
*Dicaeoma Majanthae* (Schum.) Arth., New York State  
Mus. Bull. 266: 43. 1925.

The scarcity of this rust on *Trillium* and *Brachyelytrum* arouses interest in factors that may be responsible. This rust apparently depends upon the two hosts for the completion of its life cycle. There is no indication that the urediniospores can be carried through the winter. Telia have been found within two weeks after the appearance of uredinia and no uredinia have been observed late in the fall. The two hosts may not, in most places, be growing in sufficient proximity to each other to permit the continued existence of the rust after being introduced. The fungus may be quite sensitive to environmental conditions so that periods when conditions are at all favorable may be relatively short during most seasons, or favorable locations where the two hosts occur may be few in number. The aeciospores may be short lived although they are produced during a period of a month or more. At Taughannock Falls the fruiting bodies of *Darlucula filum* (Biv.) Cast. have been observed among the aecia and especially in the uredinial and telial sori. This fungus is known to be parasitic to Uredineae and may seriously interfere with the development of this *Uromyces*. Gall midge larvae, determined by New York State Entomologist, E. P. Felt,<sup>3</sup> to belong in the family Itonididae, possibly a species of *Mycodiplosis*, were commonly present among the aecia, supposedly feeding on the aeciospores. During 1922, they were very abundant, each spot having one or more of these larvae present. These mycophagous insects are capable of diminishing greatly the number of aeciospores available for inoculation. From observations made at Taughannock Falls it would seem that, when the two hosts occur near each other

<sup>3</sup> Letter of July 19, 1927.

and the rust fungus has become established on them, no one of these factors is all-important.

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#### DESCRIPTION OF PLATES

##### PLATE 15

##### *Uromyces Halstedii* on *Trillium* sp.

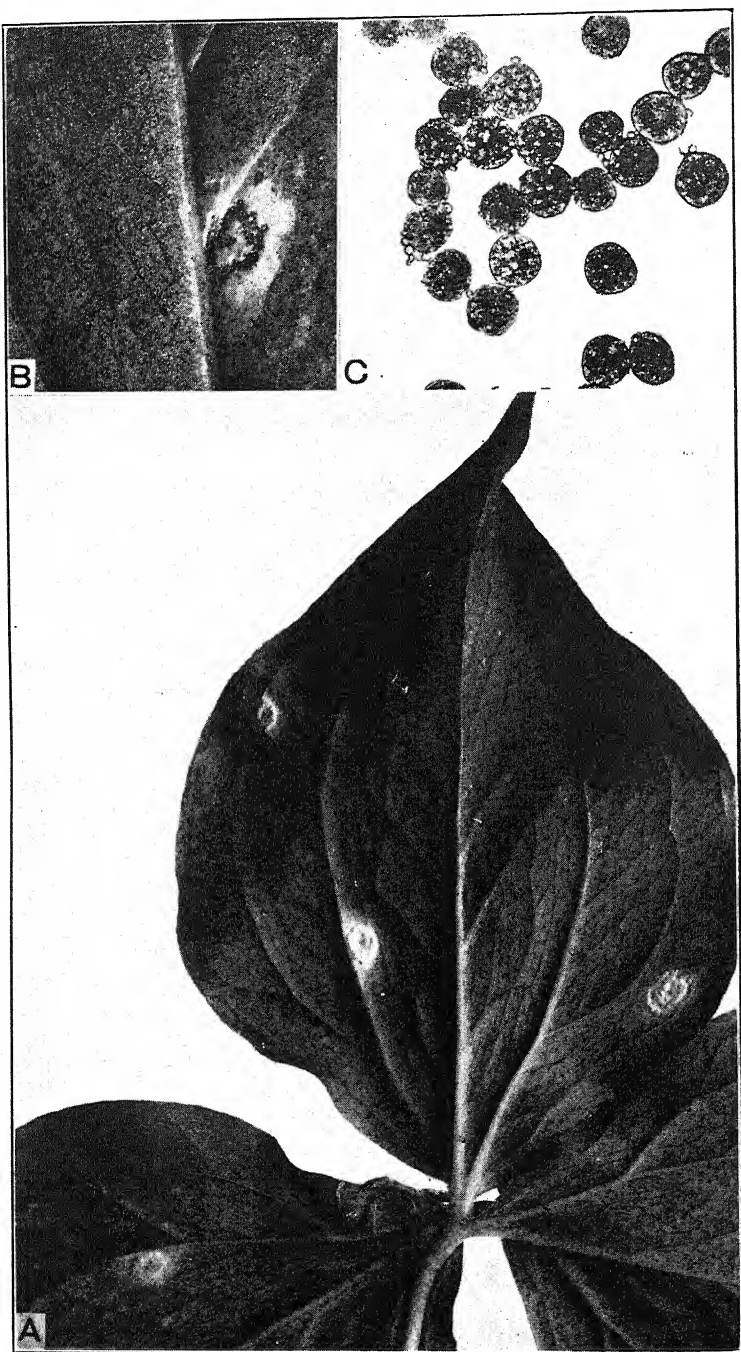
- A. Aecial lesions on lower surface of leaves of *Trillium grandiflorum*. Natural size. Photographed June 5, 1925.
- B. Aecial lesion on young *Trillium* leaf, showing aecia, pycnia, and discolored zone about aecial ring.  $\times 3$ . Photographed June 15, 1924.
- C. Aeciospores from *Trillium erectum*.  $\times 389$ . Photographed June 11, 1924.

##### PLATE 16

##### *Uromyces Halstedii* on *Brachyelytrum erectum*

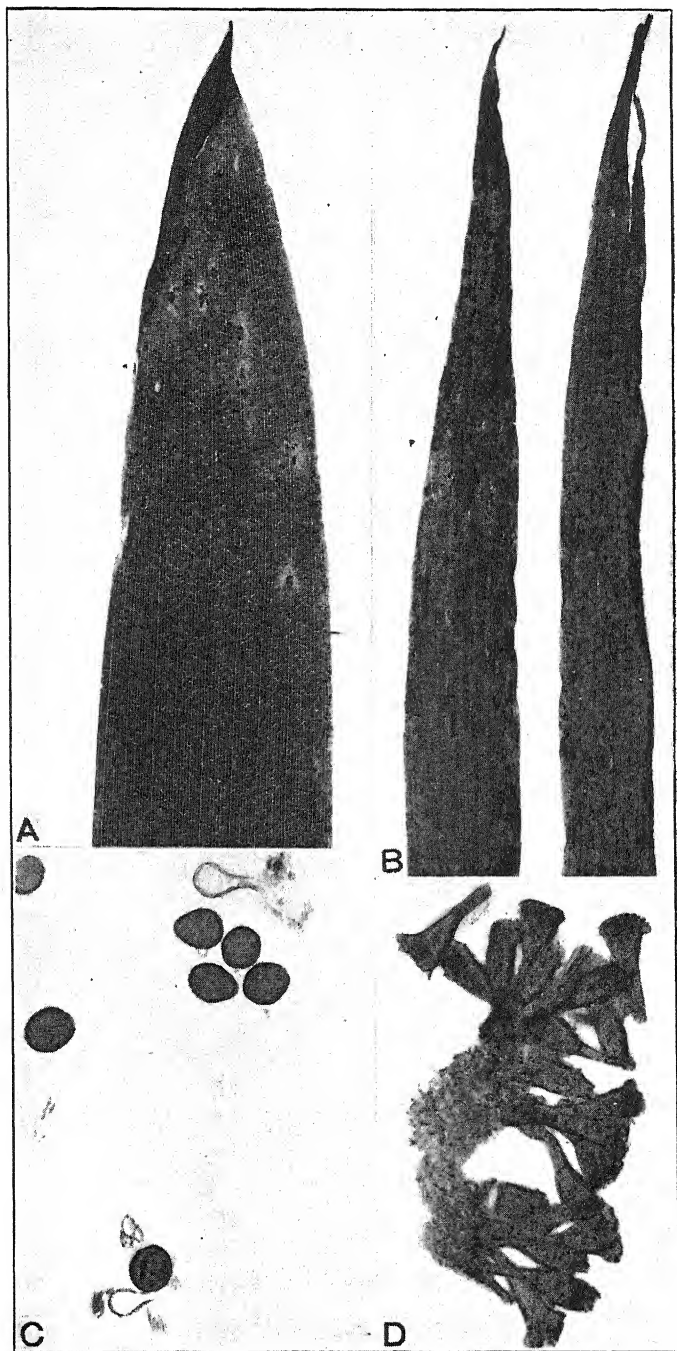
- A. Apical half of leaf showing uredinial lesions.  $\times 2.5$ . Photographed Aug. 7, 1927.
- B. Leaves showing telia and the effect of the rust on leaf tissue. Natural size. Photographed Oct. 15, 1925.
- C. Urediniospores and paraphyses.  $\times 275$ . Photographed July 4, 1922.
- D. Cluster of teliospores. The digitate projections are not well illustrated.  $\times 275$ . Photographed Oct. 15, 1925.





UROMYCES HALSTEDII





UROMYCES HALSTEDII



# THE MONILIOID SPECIES OF SCLEROTINIA

EDWIN E. HONEY

(WITH PLATES 17-19 AND 4 TEXT FIGURES)

During the past several years the writer has been engaged in a monographic study of that section of the genus *Sclerotinia* whose members possess a monilioid conidial stage. As the study progressed it became more and more essential to have as a starting point a clearer understanding of related forms not only within the genus *Sclerotinia* as recognized at present but also within related genera. This has led to the following critical remarks concerning the generic concepts in *Ciboria*, *Sclerotinia*, *Rutstroemia* and *Stromatinia* and to the establishment in this paper of the new genus *Monilinia*.

## HISTORICAL REVIEW

Much confusion exists regarding the true concept not only of the genus *Sclerotinia* but also of several closely related genera. The following historical review is an attempt to set forth in chronological sequence these various generic concepts and to evaluate them as well as possible under the present unsatisfactory nomenclatorial situation. In order to do this it has been necessary to designate the type species for several genera in cases where the authors of such genera have not done so. This is a task which would best be assumed by a committee of authorities for this particular group of fungi, and the decisions made are, therefore, offered merely as one worker's opinion on the matter.

The genus *Sclerotinia* Fuckel was first proposed by Karl Wilhelm Gottlieb Leopold Fuckel (1869: 330) in his "Symbolae Mycologicae" to include five species: *S. Candolleana* (Lév.), *S. Fuckeliana* (DeBary), *S. Libertiana* Fuckel, *S. tuberosa* (Hedw.) and *S. baccata* nov. sp. The genus was limited to fungi with rather small, long stipitate, smooth, more or less infundibuliform, fleshy, marginated especially when expanded, apothecia, which rise from a sclerotium. The cups of the

apothecia are lined by a hymenium of filiform paraphyses and elongate asci containing eight, one-celled, oblong to elliptical, hyaline spores. The species are terrestrial and vernal.

Fuckel, in the above mentioned publication, removed the first four species listed from the genus *Peziza* and added the fifth as a newly described species. Later authorities listed this fifth newly described species of Fuckel as a member of the genus *Sarcoscypha*, a genus created by Fries in 1822, but the first four are still retained in *Sclerotinia*. Fuckel did not designate a type species for *Sclerotinia*; however, by virtue of being the first binomial mentioned (Fuckel 1869: 330), *S. Candolleana* (Lév.) Fuckel becomes the type species of the genus. No illustrations were cited or given by Fuckel, but reference was given to exsiccatae. This species appears to be widespread in its distribution and has recently been reported by Wilson and Waldie (1927) as the cause of a serious oak leaf disease in Scotland and England.

The genus *Ciboria* was erected by Fuckel in the same publication (1869: 311) in which he first suggested the genus *Sclerotinia* (1869: 330), namely, his "Symbolae Mycologicae." The genus included seven species divided into two groups: the spring forms (Vernales) and the autumn forms (Autumnales). The species, listed in the order in which Fuckel gave them were as follows: (a) Vernales: *C. Caucus* (Reb.), *C. amentacea* (Balb.), *C. bolaris* (Batsch), and *C. ciliatospora* nov. sp.; (b) Autumnales: *C. rhizophila* Fuckel, *C. tremellosa* nov. sp. and *C. firma* (Pers.). Fuckel did not designate a type species; yet, because it is the first binomial species listed under the genus *Ciboria*, and because this is further supported by citations to illustrations, *C. Caucus* (Reb.) Fuckel is chosen as the type species for the genus *Ciboria* Fuckel. It is a species occurring upon the male catkins of *Populus tremula* L. and *P. alba* L. and it is said to be closely similar to but distinct from *C. amentacea* (Balk.) Fuckel. The genus, as suggested by Fuckel, included medium-sized, long stipitate fungi with a waxy smooth or pulverous surface, disk-shaped and margined and arising from twigs or grass (apparently not from a sclerotium as a sclerotium is not mentioned). The hymenium consists of paraphyses and elongate asci con-

taining eight oblong-ovate one-celled hyaline spores. In Karsten's publication of the same year, *Ciboria amentacea* (Balb.) and *C. firma* (Pers.) of Fuckel were placed in section V, Phialea of the old genus *Peziza*, the other five species used by Fuckel in the erection of the genus *Ciboria* not being listed.

As is also true in the case of *Sclerotinia*, Karsten, in his "Mycologia Fennica" two years later (1871), did not recognize the genus *Ciboria*, but placed the species suggested by Fuckel (1869) for *Ciboria* either in his genus *Rutstroemia* or did not list them. Karsten, who published his "Monographia Pezizarum fennicarum" in the same year (1869) that Fuckel created the genus *Sclerotinia*, divided the old genus *Peziza* into twenty-five sections. In section V, Phialea, Karsten recorded two of the species, *Peziza tuberosa* (Hedw.) Bull. and *Peziza Sclerotiorum* Lib. (= *S. Libertiana* Fuckel), which Fuckel included in his new genus *Sclerotinia*. Two years later Karsten, in his "Monographia Fennica" (1871), did not recognize the genus *Sclerotinia* but created, among many others, the new genus *Rutstroemia* (p. 105). Under this new genus were listed seven species: *R. bulgarioides* (Rab.) Karst., *R. tuberosa* (Bull.) Karst., *R. amentacea* (Balb.) Karst., *R. homocarpa* Karst., *R. Curreyana* (Berk.) Karst., *R. macilenta* Karst., and *R. firma* (Pers.) Karst. respectively. Since no type species was designated, *Rutstroemia bulgarioides*, the first binomial species named, becomes the type species. Karsten gave citation to exsiccatæ for this species. The genus was described as possessing elongate stipitate, glabrate apothecia, cup-like to infundibuliform in shape and somewhat closed at first, in many cases arising from a sclerotium. The hymenium was composed of cylindrical asci containing eight oblong ellipsoid or ovoid one-celled, nonguttulate spores, very rarely with slight false septation, and filiform paraphyses slightly curved at the ends. This genus included large or medium-sized fungi, of a pale brownish or sooty color.

In 1885 Karsten published his "Revisio monographia atque Synopsis ascomycetum in Fennia hucusque detectorum," in which he discarded his genus *Rutstroemia* stating that it was synonymous in part with *Sclerotinia* Fuckel (p. 123) and *Ciboria* Fuckel (p. 143), which he here recognized for the first time.

The type species of the genus *Rutstroemia* as suggested by Karsten (1871: 105), namely *Rutstroemia bulgarioides* (Rab.) Karst. was placed in his "Revisio Monographica" (1885: 124), in the genus *Chlorosplenium* Fries, it being a synonym of *C. lividum* (Alb. & Schw.) Karst. Under the genus *Sclerotinia* Fuckel (p. 123-124), Karsten listed four species occurring in Finland: *S. tuberosa* (Hedw.) Fuckel, *S. Libertiana* Fuckel, *S. Curreyana* (Berk.) Karst., and *S. adusta* Karst. respectively. The synonymy given here also shows that all four species had previously been placed by Karsten in his genus *Rutstroemia* which he here discarded. In this same paper, Karsten placed one species (occurring in Finland), *C. firma* (Pers.) Fuckel, which had also been included originally under his genus *Rutstroemia*, in the genus *Ciboria* of Fuckel.

Summarizing the above data we find the two genera, *Ciboria* and *Sclerotinia*, established by Fuckel, the only difference being in the origin of the apothecia from sclerotia (true sclerotia) in the case of *Sclerotinia* and the entire absence of sclerotia in the case of *Ciboria*. Thus far, all the species included in the genus *Sclerotinia* arise from a sclerotium composed entirely of fungous tissue and of a more or less definite size and shape. None of the fruit or tissue inhabiting pseudosclerotial forms have as yet been included in this genus. The genus *Rutstroemia* is apparently nonvalid but is synonymous in part with *Sclerotinia* and in part with *Ciboria*.

The next work of importance on the Discomycetes, that of Saccardo, "Conspectus generum Discomycetum hucusque cognitorum" (1884), subdivided the families on the basis of color, form and degree of septation of the spores. A few large genera with a number of subgenera were adopted. *Sclerotinia* Fuckel (= *Rutstroemia* Karst. *pro parte*) was given generic rank (1884: 216) with *S. tuberosa* (Hedw.) Fuckel cited as representative in the Hyalosporae Sacc. of the group Pezizeae Fuckel. Saccardo stated that the apothecia arose from sclerotia. *Ciboria* Fuckel (= *Rutstroemia* Karst. *pro parte*) was placed in the Hyalosporae Sacc. of the group Dermateae Fries (1884: 248). *Rutstroemia* Karst. was mentioned only as synonymous in part with *Ciboria* and in part with *Sclerotinia*. Five years later Saccardo published



on the Discomycetes in his "Sylloge Fungorum" (1889) and while basing his treatment of them on the "Conspectus" (1884), nevertheless he drew generic limits more closely, raised many subgenera of the "Conspectus" to generic rank and added new genera. In the "Sylloge Fungorum," *Sclerotinia* Fuckel (1889: 195) and *Ciboria* Fuckel (1889: 201) were given generic rank, *Rustroemia* Karst. being cited as synonymous. Karsten's type for the genus *Rustroemia*, *R. bulgarioides* (Rab.) Karst. was recognized as synonymous with *Chlorosplenium lividum* (Alb. & Schw.) Karst. (1889: 319), apparently following Karsten, as above mentioned, in his "Revisio Monographica" (1885).

In 1885 Boudier published a preliminary paper, "Nouvelle classification naturelle des Discomycetes charnus," in which he endeavored to group in a natural manner the species studied by him in the fresh state. In this paper (1885: 115-116) Boudier recognized the genus *Ciboria* Fuckel and subdivided it into three subgenera as follows:

(1) Subgenus *Sclerotinia* (Fuckel) Boud., apothecia arising from a sclerotium, with *P. tuberosa* Hedw., *subularis* Bull., *Duriaeana* Tul. and *Curreyana* Berk., listed in this sequence.

(2) Subgenus *Stromatinia* Boud., apothecia arising from an effuse stroma, with *P. Rapulum* Bull. listed first and then *pseudotuberosa* Rehm as representatives.

(3) Subgenus *Ciboria* (Fuckel) Boud., apothecia arising from neither an apparent stroma nor a sclerotium for *P. Caucus* Rebm. and *amentacea* Balb.

In 1907 Boudier published his work entitled "Histoire et Classification des Discomycetes d'Europe." The subgenera *Ciboria*, *Sclerotinia*, and *Stromatinia* of his earlier work (1885: 115-116) were here given generic rank, the three genera composing the tribe Ciborieae. Boudier recognized *Ciboria* Fuckel and *Sclerotinia* Fuckel in practically the same sense that Fuckel originally described them; however, he defined them somewhat more carefully. The characters of the asci and paraphyses are the same in all three genera: *Ciboria* Fuckel, *Sclerotinia* Fuckel and *Stromatinia* Boudier. *Ciboria* possesses ovoid nonseptate spores; the apothecia arise from dead twigs without trace of stroma or sclerotium. *Sclerotinia* possesses ovoid simple, guttu-

lated spores, rarely septate; the apothecia arising from a sclerotium. *Stromatinia* resembles *Ciboria* and *Sclerotinia* in the character of the apothecium, but is distinguished by the origin of the apothecium from a stroma which covers the organs on which it develops, but which is not differentiated into a sclerotium. This stroma may be white when it is internal, or black when spread over the surface. The species included in *Stromatinia* occur mostly on grains or fruits. The genus *Rutstroemia* Karst. was not mentioned by Boudier, but he included the type of the genus, *Rutstroemia bulgarioides* (Rab.) Karst. (= *Chlorosplenium lividum* (Alb. & Schw.) Karst.) in the genus *Ciboria* as *Ciboria livida* Alb. & Schw. *Ciboria Caucas* (Reb.) Fuckel, the type species of *Ciboria* Fuckel, as would be expected, was here placed in *Ciboria*, it being the first species listed. *Sclerotinia Candolleana* Lév. as well as *S. tuberosa* Hedw., *S. Libertiana* Fuckel, *S. Curreyana* Berk., *S. Duriaeaana* Tul. and others with a true sclerotium were listed under *Sclerotinia*. The type species for the genus *Stromatinia* Boud. would appear to be *Stromatinia Rapulum* (Bull.) Boud. (= *Geopyxis Rapulum* Bull.). This fungus forms its black stroma on the rhizomes of its host plant similar to *Stromatinia Paridis* Boud., the second species listed here by Boudier. It was the first species listed by Boudier (1907: 108) who gave citations to illustrations and to previous Latin description by Saccardo, in which Saccardo published this species under the name *Geopyxis Rapulum* Bull. Thus we see Boudier transferred *G. Rapulum* Bull. from the genus *Geopyxis* Pers. and apparently used it as the type of the genus *Stromatinia* Boud.

In 1887 William Phillips published his "Manual of the British Discomycetes" in which he divided the group Pezizae into two series, Nudae and Vestitae, on the basis of the external features of the apothecium. *Sclerotinia* Fuckel and *Ciboria* Fuckel were here arranged as subgenera of the genus *Hymenoscypha* Fries in Series I, Nudae. The subgenus *Sclerotinia* was described as possessing long-stemmed apothecia arising from sclerotia. The following species were included in this subgenus: *H. tuberosa* (Bull.) (= *Sclerotinia tuberosa* Fuckel), *H. Candolleana* (Lév.) (= *S. Candolleana*), *H. sclerotiorum* (Lib.) (= *S. Libertiana*

Fuckel), *H. Duriaeaana* (Tul.) (= *S. Duriaeaana* Tul.), *H. filipes* Phill. (= *S. filipes* Phill.), *H. Curreyana* (Berk.) (= *S. Curreyana* (Berk.) Karst.) and *H. ciborioides*. It will be seen from this list that only species of *Sclerotinia* possessing a true sclerotium were included in this list. Phillips distinguished *Ciboria* Fuckel from *Sclerotinia* Fuckel by the character of its not rising from a manifest sclerotium. Eleven species were included in *Ciboria*. M. C. Cooke in his "Handbook of Australian Fungi" (1892: 263) listed one species each for *Sclerotinia* and *Ciboria*, evidently following the example of Saccardo in his "Sylloge Fungorum" (1889). Rehm (1887-1894) in "Rabenhorst's Kryptogamen-Flora" divided the family Helotiaceae into two subfamilies, the first of which was divided into four tribes, Pezizelleae, Cyathoideae, Hymenoscypheae and Sclerotieae. The tribe Cyathoideae was again subdivided into two subtribes, one of which was Ciborieae. It was in this group, Ciborieae, that Rehm gave recognition to *Ciboria* and *Rutstroemia* and *Chlorosplenium* which he separated by the following key.

Apothecia upon highly colored substratum.

Spores one-celled..... *Chlorosplenium*.

Apothecia upon scarcely colored substratum.

Spores one-celled..... *Ciboria*.

Spores by septation finally two- to four-celled..... *Rutstroemia*.

Rehm included here *C. Caucus* (Rebent.), the type species for *Ciboria* as first used by Fuckel, and his limits to the genus were quite similar to those of Fuckel. *Rutstroemia*, as first described by Karsten, has here been modified to include but one of the species originally included in the genus by Karsten. Rehm must have used *Rutstroemia firma* (Pers.) Karst. as the type in his conception of the genus. He stated that the spores were elliptical, or spindle-shaped, at first unicellular, later becoming two to four-celled, due to septation. These characteristics along with that of the commonly colored tips of the paraphyses formed the principal distinction between his concept of the genera *Ciboria* and *Rutstroemia*. The type of *Rutstroemia* Karst. (Karsten, 1871: 105), *R. bulgarioides* (Rab.) Karst. (= *Chlorosplenium lividum* (Alb. & Schw.) Karst.) was placed by Rehm in the Bulgariaceae in the genus *Ombrophila* as *O. strobilina*

(Alb. & Schw.) Rehm. From these data it would appear that the genus *Rutstroemia* Karst. (*pro parte*) as used by Rehm is not a good genus, as the species, *R. bulgarioides* (Alb. & Schw.) Karst., was admitted by Rehm to belong to an entirely different group. Applying the principle of types, if the distinction which Rehm made, namely, of spore shape and septation and the generally occurring coloration of the tips of the paraphyses, is worthy of separating these species from *Ciboria*, it would then seem necessary to apply a new name to this genus rather than that of *Rutstroemia* Karst. Durand (1900: 492) has apparently preferred to accept the genus *Rutstroemia* Karst. as emended by Rehm.

*Sclerotinia* was the only genus included in the tribe Sclerotieae by Rehm (1887-1894: 800-824). Rehm divides the genus *Sclerotinia* into two subgenera: I. *Stromatinia* Boud. restricting it to those species in which the sclerotia were formed within fruits (1887-1894: 804), and II. *Eusclerotinia*, where the sclerotia were embedded in the tissues of stems or leaves. All the monilioid species of *Sclerotinia* known to Rehm were described under the subgenus *Stromatinia* (Boud.). Rehm described in this tribe, in the following sequence: *Sclerotinia Urnula* (Weimn.) Rehm, *S. Oxycocci* Wor., *S. Rhododendri* Fischer, *S. baccarum* (Schröt.), *S. megalospora* Wor., *S. Padi* Wor., *S. Aucupariae* Ludw., *S. Cerasi* Wor., *S. Mespili* Wor., *S. pseudotuberosa* (Rehm), and *S. Juglandis* (Preuss.). *Sclerotinia Candolleana* (Lév.) Fuckel, type of the genus *Sclerotinia* Fuckel, as well as *S. tuberosa* (Hedw.), *S. Libertiana* Fuckel, and others with true sclerotia, was listed in the subgenus *Eusclerotinia*. In addition to these two subgenera Rehm gave two doubtful species, *S. Rapulum* (Bull.) and *S. Tuba*. *Sclerotinia Rapulum* was the type species of the genus *Stromatinia* and the removal of this species from that subgenus would cast doubt on the validity of the use of the name *Stromatinia*. It should be noted that Rehm's criterion for separating these two subgenera was not on the morphology of the fungi involved but on the part or organ of the host affected.

Schröter (1893), in Cohn's "Kryptogamen-Flora von Schlesien," recognized for the most part the same families in the Pezizineae as Rehm (1887-1894). Schröter, however, did not

recognize the genus *Rutstroemia*, citing certain species of this genus, given by Rehm (1887-1894: 763-769), as synonyms of *Ciboria*. He also listed certain species at first included by Karsten (1871) in *Rutstroemia* Karsten as synonyms of *Sclerotinia*. Doubtless, Schröter either did not believe that septation of the ascospores was a good generic character or he recognized the nonvalidity of the genus *Rutstroemia* or he held both views. At least he did not separate the species possessing unicellular ascospores from those with multicellular ascospores. His description of the genus *Ciboria* Fuckel (1893: 60) stated that the ascospores were ellipsoidal, or ellipsoidal-spindle-shaped, one-celled, often at maturity becoming more than one-celled due to septation. Schröter described the genus *Sclerotinia* as possessing one-celled ascospores and separated *Sclerotinia* from *Ciboria* by the fact that it arose from a sclerotium, a structure which was lacking in *Ciboria*. Thus it appears that Schröter has given more nearly Fuckel's original limits to the genera *Sclerotinia* and *Ciboria*, which were also those eventually adopted by Karsten (1885), Phillips (1887), and Saccardo (1889).

Like Rehm, Schröter (1893: 63-67) divided the genus *Sclerotinia* into two groups: (a) Sclerotia developed on leaves and stems, which included *S. Candolleana* Lév., *S. sclerotiorum* (Lib.) and others with true sclerotia, (b) Sclerotia developed in fruits which included *S. baccarum* Schröter and other monilioid species of *Sclerotinia*. *Rutstroemia bulgarioides* Karst., the type of the genus *Rutstroemia* Karst., was placed in the genus *Ombrophila* Fries, as *O. strobilina*, which was the same disposition of it as that made by Rehm (1887-1894: 482).

In 1894 Schröter began to write the Discomycetes for Engler and Prantl, but on account of his death, Lindau continued the work along the same general outline that Schröter had initiated. The treatment of the genera of the Pezizaceae by Lindau seems to be closer to Rehm's than to Schröter's earlier treatise (1887-1894). Lindau used the same key as that used by Rehm to separate the genera *Chlorosplenium*, *Ciboria*, and *Rutstroemia* and he also used the same character, presence of sclerotia, to separate *Sclerotinia* from this group of three genera. The limits of *Ciboria* Fuckel and *Rutstroemia* Karst., as well as the sub-

division of the genus *Sclerotinia* Fuckel into two subgenera: (1) *Stromatinia*, and (2) *Eusclerotinia*, were the same as those of Rehm (1887-1894). Furthermore, Lindau, like Rehm (1887-1894: 482), listed *Rutstroemia bulgarioides* Karst. (= *O. strobilina* (Alb. & Schw.) Rehm) under the genus *Ombrophila* Fries. It is apparent that while both Rehm (1887-1894) and Lindau (1896: 197-199) have used the name *Stromatinia* as a subgenus they have altered the original concept of Boudier. The basis on which these authors made the division of the genus *Sclerotinia* does not appear to be a logical one, nor does it necessarily show the natural phylogenetic relationship.

Elias J. Durand published a paper on "The classification of the fleshy Pezizineae with reference to the structural characters illustrating the basis of their division into families" in 1900. It is sufficient to mention here that Durand recognized the genus *Rutstroemia*, making the primary basis of its separation from *Ciboria* and *Sclerotinia* on the septation of the ascospores, in this respect following Rehm and Lindau. Whether Durand placed *Rutstroemia bulgarioides* (Rab.) Karst., the type of *Rutstroemia*, in the genus *Ombrophila* as Rehm did, or retained it in *Rutstroemia* cannot be ascertained from his paper, but it is very likely that he followed Rehm in this regard. *Ciboria* was separated from *Sclerotinia* on the absence of a sclerotium.

#### DISCUSSION

It is the purpose of this historical review to determine the present status of the genera herein mentioned. At the present time the situation in respect to codes of nomenclature, which might be followed, is more or less in a state of chaos. It is generally felt that the international code should be revised; but, on the other hand, it is not felt that the American code is entirely satisfactory. Thus it seems best to follow a somewhat middle ground. For that reason the international code is followed, but recognition of the principle of types is also applied. As has been stated (Hitchcock, 1922, and Sprague, 1923), while the principle of types is not included in the international code, yet it is not opposed to the principles of that code and may be well applied along with it. This attitude seems to be generally

held by many European and American botanists of either School, and it is probable that the principle of types and provisions for determining types will be adopted at some future "International Botanical Congress."

In applying the principle of types (Bull. Torrey Club 34: 167-178) to the case in hand we find that both *Ciboria* and *Sclerotinia* have priority over *Rutstroemia*, that the type species of *Rutstroemia* is found to belong to an older genus *Ombrophila*, that the other species should be transferred to either *Ciboria* or *Sclerotinia*, and that Karsten who created *Rutstroemia* has himself abandoned the genus. However, it has been customary in the past for mycologists to retain the abstract idea of a genus even though all binomial species including the type may be found to be incorrectly described and therefore transferred to another genus or to other genera. This principle, however, is contrary to the type-concept, and instead of retaining the abstract idea of a genus or species, which was incorrectly described, and therefore a description of something which in reality never existed, in which to place any species which might later be found to conform to it, the genus is rejected because it is not so associated with a species previously or simultaneously published. In addition to Karsten (1885), Saccardo (1884 and 1889), Boudier (1885 and 1907), Phillips (1887), Cook (1892) and Schröter (1893) did not recognize the genus *Rutstroemia* as valid. Rehm (1887-1894) apparently finding that *Ciboria firma* (Pers.) Fuckel (1869) had been included in the original list under *Rutstroemia* by Karsten and also finding that it differed from the other species in ultimately possessing at maturity a septation of the ascospores, modified the original limits of *Rutstroemia* Karst. to include only those species (otherwise similar to *Ciboria*) possessing 2- to 4-celled ascospores. Lindau (1896) and Durand (1900) have apparently followed Rehm in this particular. To the writer the genus *Rutstroemia* Karst. seems to be nonvalid for the following reasons:

(1) The type species (*R. bulgarioides* (Rab.) Karst.) of *Rutstroemia* has been shown to be a member of another older genus (*Ombrophila*).

(2) Although *R. firma*, which had previously been included in

*Ciboria* by Fuckel, was one of the species listed (last) under *Rutstroemia*, yet it is not the type species.

(3) The original description of *Rutstroemia* Karst. agrees with *R. bulgarioides* (Rab.) Karst., its type species, in having 1-celled ascospores.

(4) *Rutstroemia firma* and any other species possessing ascospores of more than one cell, included under *Rutstroemia* Karst. by Rehm or others, do not agree with the original generic description of *Rutstroemia*, and, therefore, the genus *Rutstroemia* has no binomial representing it which is in harmony with the original description, whereas the genus *Rutstroemia* Karst. as recognized and amended by Rehm calls for 2- and 4-celled ascospores. All representatives meeting with the original description of Karsten have been removed, due to priority, to the genera *Ciboria* and *Sclerotinia*.

It would seem to the writer that if a group, such as Rehm separated from *Ciboria* Fuckel on the basis of the septation of the ascospores, is worthy of recognition, a new generic name other than that of *Rutstroemia* Karst. should be used.

The type species of *Stromatinia* is *S. Rapulum* (Bull.) Boud. The exact nature of the sclerotium is difficult to determine from the literature. Bulliard (1798: *Pl.* 485, *fig.* 2) illustrated three apothecia but no attachment. Cooke (1879: *Pl.* 50, *fig.* 197) figured four complete apothecia and two in longitudinal section but no attachment. Persoon (1801: 658-659) and Fries (1822: 59) did not mention the attachment. All the above named authorities spoke of long filiform, tortuous stipes, deeply embedded in the ground and among leaves, which no doubt accounted for the failure to either find or illustrate the attachment. The older authorities may have failed to see the importance of the substratum. Boudier (1905-1910: 3: *Pl.* 478) illustrated the apothecia attached to a rhizome which greatly resembles that of our common Solomon's seal, *Polygonatum biflorum* Walt. and *P. pubescens* (Willd.) Pursh. In volume four of his "Icones," Boudier (1905-1910: 4: 277-278) stated that *Stromatinia Rapulum* (Bull.) Boud. had no sclerotium but arose directly from the rhizomes of *Polygonatum multiflorum* or *P. vulgare* which it rotted and replaced by a black and fragile stroma. Boudier



apparently had the idea of a stroma enveloping the organs upon which it was developed, but one not differentiated into a definitely shaped separate structure or sclerotium. Whether this stroma was a mixture of host and fungous tissues or was merely a layer of pure fungous tissue developed upon the surface of the rotted host is not so clear from the description. The writer, during the spring of 1925 collected a *Sclerotinia* occurring upon the rhizomes of *Polygonatum pubescens* (Willd.) Pursh. in Cascadilla Gorge, Ithaca, New York. The apothecia strongly resemble those of *Stromatinia Rapulum* (Bull.) Boud. and it is the opinion of not only the writer, but others, that this is the same fungus Durand described as *Sclerotinia Smilacinae*, mistaking the host, *Polygonatum*, for *Smilacina racemosa*. Durand stated (1902: 462) that the sclerotia were small, 1-2 mm. in diameter, irregularly spherical, aggregated and sometimes coalesced into a thin crust-like mass, 1-2 cm. in diameter. This crust-like mass may be of a nature similar to the stroma to which Boudier referred. As it is not a pseudosclerotium but really an aggregation of small true sclerotia, it appears to the writer that *Stromatinia* may be synonymous with *Sclerotinia* Fuckel, in the restricted sense. In fact it is possible that *S. Smilacinae* Durand is the same species as *Stromatinia Rapulum* (Bull.) Boud. The writer has not had an opportunity to study *Stromatinia Rapulum* (Bull.) Boud. to determine the exact nature of its stroma, but it is hoped that this may be done in the near future. Of the remaining species placed in the genus *Stromatinia* by Boudier, possibly half are known to possess pseudosclerotia and monilioid imperfect stages. These will be discussed later.

We still have to deal with the genera *Ciboria* Fuckel and *Sclerotinia* Fuckel. The presence of the sclerotium separates the latter from *Ciboria* in which the apothecia arise directly from a mycelium in the absence of a sclerotium. DeBary (1887: 499) defined a sclerotium as a "pluricellular tuber-like reservoir of reserve material forming on a primary filamentous mycelium from which it becomes detached when its development is complete, usually remains dormant for a time and ultimately produces shoots which develop sporophores at the expense of the reserve material." This is the type of structure found in all the

first four species originally included by Fuckel (1869: 330) in the genus *Sclerotinia*. There is no doubt that the true sclerotium, that is, a sclerotium composed entirely of fungous tissue, not a mixture of host and fungous tissue (pseudosclerotium, Atkinson 1905), was the concept on which Fuckel based the genus *Sclerotinia*. The etymology of the name indicates that this is the important structure. Now it is easy to conceive of gradients all the way from the true sclerotial type to the simpler types, included in *Ciboria*, in which the apothecia arise from a mycelium having no apparent sclerotia. Among these intermediate stages would fall the group which at a later date Woronin and others included within the genus *Sclerotinia*. But at either end of the line of development, either between the forms with a pseudosclerotium and those with a true sclerotium or between the forms with a pseudosclerotium and merely a mycelium, it is possible for intergrading forms to occur. This structure is homologous throughout the group regardless of the form or composition it may assume. To the writer, the phylogenetic importance of the presence or absence of the sclerotium, which is merely the one extreme of a stromatic gradient possessed by a group of fungi exhibiting a very close relationship by the similarity of the external and internal characters of their apothecia, should not be given undue significance. It has served as a convenient, though possibly not a definite, means of designating certain groups of fungi. Now that our knowledge has extended to other species this means of separation is less satisfactory and we are more nearly able to judge relationships. Thus, for example, Fuckel created two groups, *Ciboria* and *Sclerotinia*, not being acquainted with members of a third group which later became known and which Woronin (1888) and Schröter (1893) included in the genus *Sclerotinia* and which some later authors have included in *Stromatinia* along with other species. It will be noticed that this division of genera made by the older mycologists and followed by the later ones is based solely on the stromatic stage. The understanding of their day did not permit of any other criterion in designating the genera. Our knowledge of the life-histories of these individual species is still comparatively meager. But gradually details of the life-history

of various members of *Sclerotinia*, *Ciboria*, etc., have accumulated, due to the researches of Woronin, Brefeld, von Tavel and others, so that at present we have a much clearer idea of this group. In this group, whose ascigerous fructifications arise directly from a mycelium, a pseudosclerotium or a true sclerotium, there are at least three characters in common, namely, the apothecium, microconidia, and mycelium. Von Tavel (1892: 105) has remarked about the presence of these same characters in this group of fungi. Members within the group differ principally by the absence or presence or by the type of the macroconidial stage. And again, von Tavel as early as 1892 (pp. 105-106) separated the genus *Sclerotinia* into subgroups on the basis of the conidial stage. He referred to one group as having given rise only to microconidia, a second group as having produced a *Botrytis*-like conidial stage (he apparently supposed *Botrytis cinerea* to be the conidial stage of *Sclerotinia Fuckeliana*) and a third group as having possessed besides microconidia, chlamydospores (he mentioned *S. baccarum* and *S. Vaccinii*).

The external characters of the apothecia of these three groups, as has been mentioned by Boudier (1907) and others, are so nearly alike that they are almost indistinguishable unless their attachment is known. Even with their attachment it is often difficult to determine to which group they belong until after a very careful examination. Durand (1900: 463) has studied thin sections of the apothecium of members of this group and used the internal characters of the sterile layers of the cup as important distinguishing characters to separate the fleshy *Pezizineae*. He used these internal structures to separate the different families. From this work it seems, therefore, that not only the exterior but also the internal morphology of the apothecium is indistinguishable in the three groups under discussion. Microconidia are present in all three groups. It is unnecessary to discuss the nature, morphology, and rôle of the microconidia here; but let it suffice to say that they correspond precisely, in all three groups, so far as known, in regard to general shape, size, internal and external characters and method of being borne. The next character to consider is that of the attachment. It is a vegetative structure. In *Ciboria* this structure may be indi-

cated as loose vegetative mycelium for, according to the concept of Fuckel, the apothecia arise directly from the host, or substratum from a mycelium which is not aggregated in the substratum. This is probably the simplest type of apothecial attachment. Then under the concept of the sclerotium as used by Woronin (1888) with the species of *Sclerotinia* occurring on *Vaccinium* or by Aderhold and Ruhland (1905) in their description of *Sclerotinia laxa*, *S. cinerea* and *S. fructigena*, we have a pseudosclerotium. In this we have a mycelium invading the outer tissues of the substratum and so permeating the tissue as to be a compact mass of both host and fungous tissue. To a somewhat similar structure in the genus *Balansia* Atkinson (1905) has applied the term *pseudosclerotium*. An important and almost constant character of these so-called pseudosclerotia, found in the fruit inhabiting species of *Sclerotinia*, is a definite blackened layer (cortex or rind) of heavy pseudoparenchymatous cells which form a protective layer on all exposed surfaces (both inner and outer) of this compact mass of host and fungous tissue. The interior (medulla) of these pseudosclerotia is as a rule whitish, the same as we find in a true sclerotium except for the dead host cells included. As the writer will show in the monographic paper, referred to above, he considers the pseudosclerotium in this group to be commonly comparable and analogous (probably homologous) to the entostroma usually recognized in the stromatic Sphaeriales. In the third group (Eusclerotinia) we find the mycelium in the form of a compact mass of pure fungous tissue such as is defined as a sclerotium by DeBary. The structure is essentially the same, namely *mycelium*, which is merely modified to meet the needs of the fungus or to comply with the restrictions placed upon the fungus by the host plant. That species which normally produce true completely differentiated sclerotia of pure fungous growth, may also have a mixture of host tissue as well as the pseudosclerotia is quite possible. *Sclerotinia sclerotiorum* and *S. Trifoliorum* oftentimes form sclerotia within the partially excavated chambers in the pith of their host plants and it is possible that they envelop cells of the host plant occasionally. Boudier (1907: 13) stated that one might very often find cells of the host plant, cells which had

been enveloped by the mycelium with the sclerotia, and cited specifically the case of *Sclerotinia Curreyana* with the sclerotia of which the star-shaped cells of the pith of the host were quite generally found. It appears evident, however, that inclusions of host cells within the sclerotia in this group of *Sclerotiniace* is purely an accidental phenomenon and not the constant typical condition characteristic of most of the fruit inhabiting group which form typical pseudosclerotia. The sclerotium is nothing more than a vegetative mycelial structure (stroma) which grades into a pseudosclerotium and into a loose vegetative mycelium on the one hand and a true sclerotium on the other. It is commonly believed that the function of the sclerotia is to produce apothecia. Since sexuality is assumed to be present in the apothecia arising from these sclerotia, the tendency has been to assign too great a significance to the sclerotium as a structure itself, rather than to the mycelium composing it. The fundamental function of the sclerotium is a vegetative food storage organ which is capable of overwintering or undergoing adverse conditions. In the genus *Ciboria* we have apothecia arising direct from a loose mycelium, sclerotia being absent, so there is no reason to ascribe a peculiar sexual nature to the vegetative, mycelial structure known as a sclerotium. The mycelium in the case of *Ciboria* apparently is capable of overwintering, undergoing the environmental and seasonal vicissitudes and of securing sufficient nourishment for apothecial production without the formation of a special reserve food supply and protective coating such as the typical sclerotium offers. Even Boudier has inferred that it was the function of the sclerotium to give rise to apothecia, but it does not seem to the writer that the sclerotium, as a structure, should be emphasized in a consideration of sexuality but rather the mycelium which is present in *Ciboria*, *Stromatinia*, or *Sclerotinia* regardless of whether the sclerotium or pseudosclerotium is present. A further discussion of the nature of the sclerotium and pseudosclerotium in the genus *Sclerotinia* will be given in the monographic paper soon to appear.

Thus we see that while sclerotia are present in certain members of this group of fungi now placed in *Ciboria*, *Stromatinia*, *Rutstroemia* or *Sclerotinia*, depending upon the authority, yet they

are not indispensable since they are entirely lacking in other species. Nevertheless, the sclerotium has been used in the past and it would seem out of question to ignore this structure entirely in taxonomy at present. Recognition must be given to the fact that, if the presence or absence of the sclerotium is used as a means of separating groups, there are marginal forms which might be of doubtful nature. This may be emphasized because of great variation within even a single species, as in the following example. The writer has collected specimens of *Sclerotinia Smilicinae* Durand which would appear to belong to *Ciboria* on the basis of manifest sclerotia. The apothecia appeared to arise directly from the rotted rhizomes, no sclerotia being evident. This is explained, however, by the fact that the sclerotia in this species are extremely small and were imbedded within the rotted rhizomes. Cultures from ascospores of this same collection gave rise to a multitude of small black sclerotia in and on the potato glucose agar. Under certain conditions the small sclerotia may aggregate and form a crust over the diseased rhizome and give the impression of a stroma. It would appear, therefore, that in order to show phylogeny and to arrange these species most satisfactorily for taxonomic purposes, the type of the attachment, whether loose mycelium, pseudosclerotium, true sclerotia or otherwise, should be supplemented by other structures produced during the life-histories of the fungi.

We still have to consider the imperfect stages occurring in the life-history of members of this group. Complete life-history studies are relatively few. There are many gaps, therefore, which research will need to fill in. However, on the basis of our present knowledge we have made a substantial start in this direction, thanks to the work of Woronin, Brefeld and others. Brefeld and von Tavel (1891: 318) stated that the ascospores of *Ciboria bolaris* Batsch and *Ciboria firma* Pers. in culture developed only a sterile vegetative mycelium except for the microconidia which were present in both. The writer also has cultured several species of *Ciboria* including *C. Caucis* (Rebent.) Fuckel and *C. amentacea* (Balb.) Fuckel with results similar to those of Brefeld and von Tavel. In nature, upon the proper host plant or substratum, these species might develop a typical

macroconidial stage; but if such occurs it is not known. It is probable that most of the species correctly attributed to *Ciboria* in the sense of Fuckel have no macroconidial stage but merely develop vegetatively into mycelium which is either scant in some species or very luxurious in other species. Certain members which Boudier placed in *Stromatinia*, for example *Stromatinia Rapulum* (Bull.) Boud. and probably *S. Paridis* Boud. and others, also lack a macroconidial stage, producing only the microconidia and the otherwise sterile vegetative mycelium, so far as known. Also certain members included by Fuckel in his genus *Sclerotinia*, as for example *S. Candolleana*, *S. Libertiana*, *S. tuberosa*, and others since placed in *Sclerotinia*, as *S. Smilicinae* Durand and *S. minor*, etc., would fall into this category. Thus we find within the various groups, based upon the absence or presence or type of sclerotium, that a subgroup may be arranged based upon the absence of a macroconidial stage.

Another group of species, included among those under consideration, has been definitely connected with macroconidial stages. The greater number of these forms are those whose imperfect stage falls in the Hypomycetous genus *Monilia* Pers. The conidia of this group are hyaline or light colored, ovate to lemon-shaped and borne in chains, commonly known as monilioid chains. Disjunctors between the conidia are common in many species while in others these structures are absent. Among species possessing this imperfect stage may be listed *Sclerotinia baccarum*, *S. Vaccinii*, *S. fruticola*, *S. laxa*, and others to be dealt with in the forthcoming monograph. Other Hypomycetous conidial stages have been definitely connected with the apothecial stages of members of *Sclerotinia* Fuckel, for example, Seaver and Horne (1918) described a species of *Sclerotinia* with a *Botrytis*-like stage. This has been termed a *Botrytis*-like stage, because, while the general shape of the conidiophore and the conidium is that of *Botrytis*, the conidium instead of being smooth becomes quite strongly roughened, and because there may be other minor differences. This is the only species known to have this type of imperfect stage but it is closely related to *Botrytis*, and a *Botrytis* stage has been connected up with *S. Ricini* Godfrey (1919). Thus *Sclerotinia Geranii* Seaver &

Horne and *S. Ricini* Godfrey might form another group or groups with botrytioid or pseudobotrytioid conidia. A *Penicillium*-like type of conidial production has been described by Maul (1894) and Rostrup (1897) for *Sclerotinia Alni* Maul. This may form still another division on the basis of the imperfect stages although there is some doubt regarding this point and it may even constitute nothing more than the microconidial stage. Possibly further observations and researches will add new members to the groups already indicated and add other types of imperfect stages which are at present unknown.

Thus we have reviewed briefly the general conditions now known to exist in that group of fungi, designated by Fuckel (1869) under the genera *Ciboria* and *Sclerotinia*, by Karsten (1871) under *Rutstroemia* and later (1885) also under *Ciboria* and *Sclerotinia* and by Boudier (1907) under the tribe Ciboriées (which includes *Ciboria*, *Stromatinia* and *Sclerotinia*). In the present state of our knowledge regarding this group of fungi a complete and final diagnosis would be premature at this time. Such a diagnosis of the genus *Sclerotinia* is being undertaken by Professor H. H. Whetzel.

To the student of the fungi reviewed above, it is apparent that the forms dealt with and recognized as properly belonging to this group are closely related. In fact they so intergrade that a sharp line of demarcation cannot be drawn between certain groups of them. Therefore there may be the tendency on the part of certain workers to merge those forms, which Boudier (1907: 105) included within the tribe Ciboriées, into one genus in a manner somewhat similar to that of Karsten when he created the genus *Rutstroemia*. If this procedure were followed in dealing with this group of *Peziza*-like fungi, *Ciboria* would have priority, and if one genus were recognized, *Ciboria* Fuckel would necessarily be the valid name to apply. Such a merging of these closely related forms is, however, not the tendency among modern mycological workers, and it appears to be untenable at present. This group of fungi appears rather to represent a subfamily, as Boudier (1907) has treated them, or a distinct family. The writer believes that the genera *Ciboria* and *Sclerotinia* should be recognized as distinct genera and that



the genus *Sclerotinia* as it is now recognized should be subdivided into either subgenera or genera on the basis not only of the nature of the stroma but also of the type of conidial fructification, grouping those forms which appear to be most clearly related phylogenetically. Genera should show phylogenetic relationships as nearly as possible, yet they are at best little more than arbitrary units. In this group of fungi the picture or configuration of the individual species or of genetic concepts is not complete or adequate when considering but one stage, such as, for example, the stromatic stage, exclusive of the apothecial or the conidial stages.

In the present paper we are not concerned directly with the disposition of other members of the genus *Sclerotinia* outside of the monilioid forms. For this reason we shall proceed to a discussion of a disposition of the monilioid species of *Sclerotinia*.

In the monographic paper which is to be published later the morphologic structures of taxonomic value which are present among the monilioid species of *Sclerotinia* will be more completely discussed. These structures of taxonomic significance are chiefly the following: the pseudosclerotium, the apothecium (including rhizoidal tufts), the conidial stage, and the microconidia.

#### CONCLUSIONS

A careful consideration of the morphological structures which are present among the monilioid species of *Sclerotinia* impresses one with the striking unity of this taxonomic subgroup. All of the members referred to the group show unmistakable evidence of close relationships, at least, closer relationship to each other than to the other members now included within the genus *Sclerotinia* and not possessing the monilioid conidial stage. It is reasonable and logical to believe that a typical *Sclerotinia* even though it may possess a pseudosclerotium and at the same time a distinct type of macroconidial stage, such as *Botrytis*, is not as closely related to each and every species of *Sclerotinia* possessing a pseudosclerotial stage and a monilioid stage as those monilioid species are to one another. The intercalation of other conidial types into the same group with the monilioid species of *Sclerotinia* breaks up the unity of the group and does not represent a natural

classification. It does not assist in the expression of phylogeny, which should be the aim of taxonomy.

Life-history studies bring out the great unity of this monilioid group in a manner much more convincing than the mere statement of the presence of certain structures such as a pseudosclerotial and a monilioid conidial stage. Other structures and physiological activities show the close relationship and unity within the group. Examples of this are blossom infection, leaf infection and mummification of fruits. All known species are virulent pathogenes giving rise to the same general type of disease symptoms (necroses). In general the individual species are believed to have a very narrow host range, *Sclerotinia fruticola*, *S. laxa* and *S. fructigena* being the greatest exceptions to this rule.

The monilioid group exhibits specialization among its members in varying degrees and different directions as would be expected among evolving organisms in a dynamic world where variation is the dominant feature and as is commonly observed in other groups and genera. Because of the lack of certain data it is difficult to trace phylogenetic tendencies in close detail but the general trend may be indicated. Thus we may consider certain species within this group as more primitive, others as more advanced morphologically and physiologically.

The question arises as to what characters are more primitive and what more specialized, and which species represent each group. It is not the purpose of this paper to answer this question for the entire group at present included within the genus *Sclerotinia*, but it would appear that *Ciboria* is more primitive than any species included in *Sclerotinia*. We might use *Ciboria*, therefore, as a starting point. Here a loose nourishing mycelium constitutes the vegetative phase; a conidial stage, so far as known, is absent. Next in rank we place those forms developing pseudosclerotia—a mixture of host and fungous growths, followed by a still more specialized stroma, the true sclerotium. Within the monilioid species of *Sclerotinia*, therefore, those forms approaching more nearly to the true sclerotial condition might be regarded as more specialized. Of the monilioid species, the Rosaceae-inhabiting group, in general, probably shows the least

specialization of the pseudosclerotium (PLATE 18). More host tissue is retained within the pseudosclerotium, and its organiza-

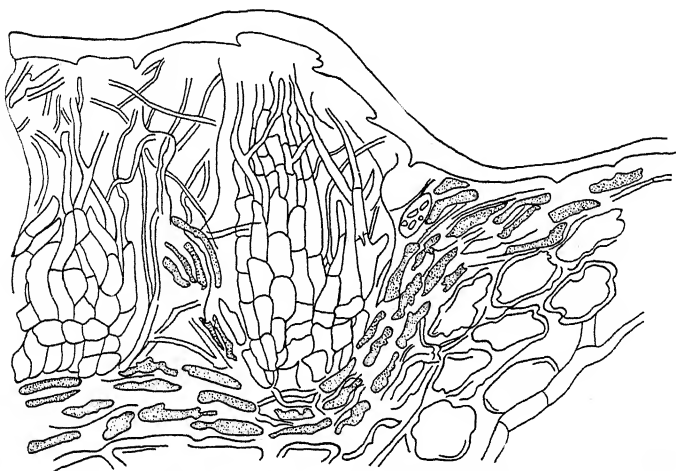


FIG. 1. Ectostroma in diseased fruit.

tion is not so definite as in the *Vaccinium*-inhabiting group. A definite ectostroma is present within the diseased fruit and

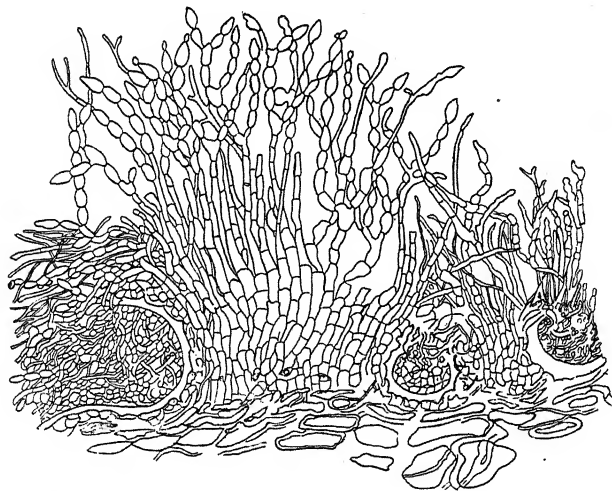


FIG. 2. Developing conidia.

commonly functions in rupturing the surface of the diseased organ and in giving rise to the conidial stage (FIGS. 1 AND 2).

Greater specialization is shown in the pseudosclerotium of the *Vaccinium*-inhabiting species and even within this group a marked advance is to be noted. Here, the pseudosclerotium consists largely of fungous growth. Cross-sections show the arrangement of the hyphae to be more nearly like that found in true sclerotia. Islands of host tissue surrounded by fungous growth are more conspicuous than the uniform presence of host tissue throughout the pseudosclerotium, as found in *S. fructicola*, for instance. The tendency is strikingly toward a more thoroughly organized fungous body. Another striking advance is the apparent tendency to segregate the conidium-producing ectostroma from the apothecium-producing entostroma and the suppression of the functioning of the ectostroma in the diseased fruit. The entostroma develops quite rapidly and the ectostroma becomes included within the entostroma, disappears or at least becomes unable to rupture the surface of the fruit and produce the conidial fructifications. Here the production of conidia is restricted for the most part, at least, to infections resulting from ascospores on shoots and leaves. Segregation and specialization are taking place. It may be considered a step toward the heteroecism described by Woronin and Nawaschin (1896) in *Sclerotinia Ledi* (= *S. heteroica*) and by Fischer (1925) in *S. Rhododendri*. The next step in specialization is represented first by the solid pseudosclerotium, then by the heteroecism as found in *S. Ledi* and *S. Rhododendri* which gives a still more complete segregation of the conidial production to one host and sclerotial and apothecial production to another host. Even a further step in pseudosclerotial development, though not accompanied by heteroecism, has been described by Schellenberg (1907: 200-201) for *Sclerotinia Ariae*, in which he stated that the pseudosclerotium was of pure fungous tissue with very few inclusions of dead host cells brought about by the development by the fungus of a closed ring, which as it grew pushed the dead tissue of the fruit equally inward and outward. He even described some small true sclerotia of this fungus being developed on the pedicels. This species would then appear to constitute the climax in the development of the pseudosclerotium within this group and indicate a border-line form with the true sclerotium producing groups

Other structures have either developed or degenerated along with the evolution of the pseudosclerotium. It is possible that the monilioid conidial fructification which we have stressed as being so very characteristic in this group may have arisen from such a form as represented by *Sclerotinia Alni*, which while lacking a conidial stage nevertheless has a pseudosclerotial stage formed within the fruit of its host. On the other hand, such species (as represented by *S. Alni* and *S. Betulae*) might be considered as representing degenerate forms. This is mere supposition on the part of the writer who has not studied intensively the species belonging to this group of *Sclerotinia*. It may be desirable to include such forms as primitive monilioid species of *Sclerotinia*, but this can be determined only by a further study and consideration of the group. Even such a form as the *Sclerotinia* developing from the pseudosclerotium of *Sclerotium bifrons* cannot be overlooked entirely. Could not this form which develops a typical composite sclerotium or pseudosclerotium within the leaves of *Populus* spp. also be a primitive monilioid form? On the basis of the presence of a pseudosclerotium alone, such a disposition would be possible, but the writer is not, at present, in a position to give his final opinion on this point. A fuller discussion of the possible basis for including these forms within the monilioid group of *Sclerotinia* will be given in the monograph. It is possible that these two last mentioned representatives can best show phylogeny by being retained as a distinct unit or units to show relationships with other groups of the *Sclerotinia* family. Therefore, while these latter forms have not been included within the monograph, referred to, the writer does not exclude them from this group of fungi.

The monilioid species of *Sclerotinia* are apparently less specialized morphologically than the botrytioid group. This conclusion is arrived at, first because of the presence of a true sclerotium in the botrytioid forms, and secondly, because of the manner of development of the conidia themselves. Botrytioid conidia are definitely, highly developed, typical conidia, formed on well developed and differentiated conidiophores. Monilioid conidia, as found in this group, are less highly specialized. In fact

Brefeld (1891: 317) did not consider the macroconidia in the monilioid species of *Sclerotinia* as true conidia but rather as chlamydospores, probably due to the acrogenous elongation and formation of the chain of spores and the intercalary development and the existence of primary and secondary walls. Von Tavel (1892: 106) also used the term chlamydospore for these spores. However, the writer considers the so-called chlamydospores as true conidia but representing a somewhat less specialized type than those of *Botrytis*. The occurrence of disjunctors within this group would indicate a further specialization in a different direction than that taken by *Botrytis*. Thus it may be concluded that the group of monilioid species of *Sclerotinia* here considered is more or less intermediate between forms possessing a loose vegetative mycelium only, but lacking a conidial stage, and those possessing a true sclerotium and a definite typical conidial stage, such as *Botrytis*. Species possessing pseudosclerotia as well as true sclerotia may lack a macroconidial stage, but they are apparently the more primitive members in their respective groups.

The distinctive characteristics of the monilioid species of *Sclerotinia* are, first, the presence of the pseudosclerotium, and second, the monilioid conidial stage. Because of its distinct unity and harmonious conformity in representing natural relationships this group is regarded as of generic rank. As shown in an earlier part of this paper the original concept of the genus *Sclerotinia* included only those forms possessing a true sclerotium. Because of the great similarity of the apothecia and the failure to recognize the significance of the pseudosclerotium, the monilioid forms along with many other divergent forms in the past have been included within the genus *Sclerotinia*. As pointed out in the historical review, no other previously proposed generic or subgeneric name or concept is valid for this group, and therefore, the writer suggests the name *Monilinia* to embrace this new genus of monilioid species of *Sclerotinia*. The name, *Monilinia*, is derived from *Monilia* (= plural of Latin *Monile* = necklace), the genus to which the characteristic conidial stage has commonly been referred. Concerning the status of the genus *Monilia* see Vuillemin (1911) and Berkhout

(1923). Not all members at present included in the genus *Monilia* are referable to *Sclerotinia* and included as the conidial stages of *Monilinia*, as might be expected in a form-genus not based upon a natural classification.

## TECHNICAL DESCRIPTION

*Monilinia* gen. nov.

*Sclerotinia* Fuckel (*pro parte*) Schroeter, Krypt.-Fl. Schlesien 3: 63-67. 1893.

*Stromatinia* Boudier (*pro parte*) Hist. Class. Discom. Eu. 108. 1907.

*Monilia* Pers. emend. Saccardo (*pro parte*) Michelia 2: 17. 1882.

*Monilinia fructicola* (Winter) comb. nov.

Apothecia small to moderately large, solitary to very many, developing from overwintered, more or less specialized composite stroma (entostroma) or pseudosclerotium which is commonly formed within the fruits of its respective hosts, closed at first, opening into a funnel- then cup-shape, sometimes becoming flat or recurved, commonly with a narrow even margin, with more or less elongated, straight or bent stipes, exterior smooth, waxy, light or dark brown, more or less hairy, especially toward the base, with or without rhizoidal tufts; asci cylindrical-clavate, somewhat thickened and rounded at the apex, with the tip or the pore frequently staining blue with iodine, eight-spored; spores elongate or ellipsoidal, ends pointed or rounded, straight or but slightly curved, unicellular, commonly possessing one to several oil-drops, guttulae, or refractive spots, generally hyaline, rarely dark, obliquely uniseriate, frequently becoming sub-biseriate; paraphyses simple or branched near the base, thread-like, septate, commonly more or less swollen at or toward the upper end, hyaline; hypothecium and excipulum distinct, prosenchymatous, generally with a pseudoparenchymatous outer layer; conidial stage common on young leaves, on shoots, on fruits, on twigs or limb cankers of the host plant; conidiophores commonly arising either from a well developed, effuse septate mycelium or ectostroma, or frequently from clustered ectostroma which ruptures the epidermis of the host organ, forming light ochre to ash grey or dark olivaceous colored sporodochia composed of more or less elongate, erect, simple or di- or trichotomously branched conidial chains; conidia (macroconidia) ellipsoidal, elongate-ellipsoidal to limoniform, rarely spherical, gen-

erally hyaline or light colored, developed acrogenously in chains upon the more or less short conidiophores, commonly separated by disjunctors or lacking these; microconidia commonly present, small, spherical, hyaline, generally containing one (more rarely two) large, round, refractive granule, borne directly or upon short clavate microconidiophores.

The type species, *Sclerotinia fructicola* (Wint.) Rehm, is the brown-rot fungus of orchard fruits commonly occurring in eastern North America and first described by Winter, from

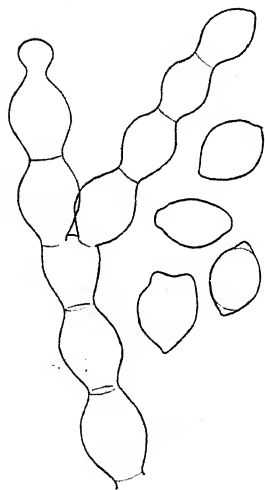


FIG. 3. Conidia.

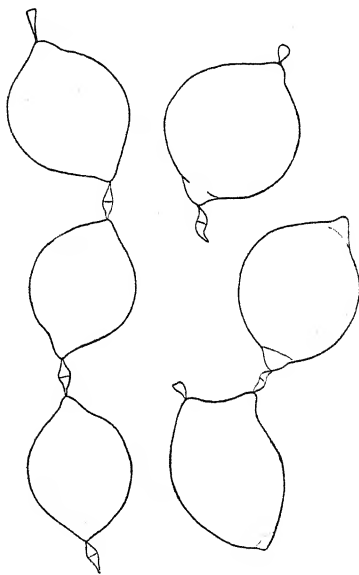


FIG. 4. Conidia.

material of the perfect stage collected by Eugene A. Rau at Bethlehem, Pennsylvania, as *Ciboria fructicola*. Type material of this species has not been seen by the writer but cotype material has been examined. The cotype material is deposited in the Eugene Rau Collections in the Office of Pathological Collections of the United States Department of Agriculture, Washington, D. C.

Typical apothecia of this species, cross-sections of the pseudo-sclerotium and the conidial stage are shown in PLATES 17, 18, and 19 respectively. The two types of monilioid conidial fructifi-



cations found in this genus are represented in FIGURES 3 AND 4. FIGURES 1 AND 2, adapted from Sorauer (1899), illustrate the development and structure of the ectostroma and of the conidial fructification in certain members of the genus *Monilinia*.

#### SUMMARY

An historical review of the closely related genera *Ciboria* and *Sclerotinia* is given.

The non-validity of the genera *Rutstroemia* and *Stromatinia* has been discussed.

Type species for these four genera have been designated following the principle of types as outlined in the American code of botanical nomenclature.

A basis for the classification of the genus *Sclerotinia*, as recognized up to the present, is indicated.

A new genus, *Monilinia*, is created to include the monilioid forms which show a genetic relationship to the genus *Sclerotinia*. *Monilinia fruticola* (Wint.) Honey (= *Sclerotinia fruticola* (Wint.) Rehm) is designated as the type species of the genus *Monilinia*.

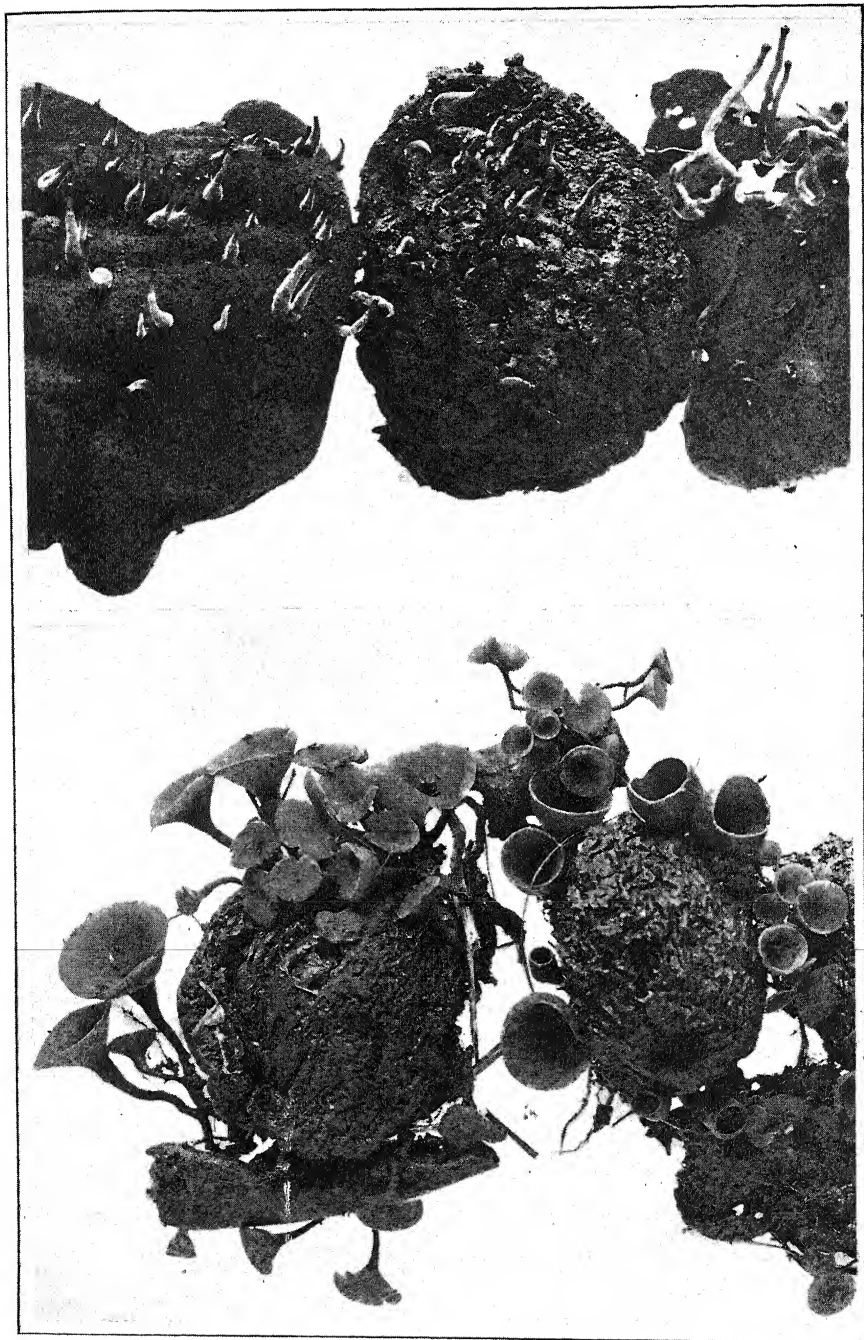
The writer wishes to express his appreciation to the various individuals to whom he has been indebted during the preparation of this article: to Professor Whetzel for the suggestion of the problem of which this is an outgrowth; to Dr. L. M. Massey for the photographic privileges of the Department of Plant Pathology of Cornell University; to Dr. C. L. Shear for the privilege of examining the cotype material; to Dr. E. M. Gilbert, Dr. G. W. Keitt, Dr. A. J. Riker and Dr. Regina Stockhausen Riker for the critical reading of the manuscript; and to Dr. Fred J. Seaver for kindly suggestions concerning the manuscript.

DEPARTMENT OF BOTANY,  
UNIVERSITY OF WISCONSIN,  
MADISON, WISCONSIN.

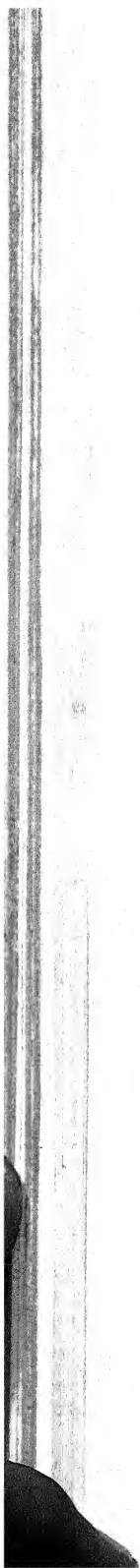
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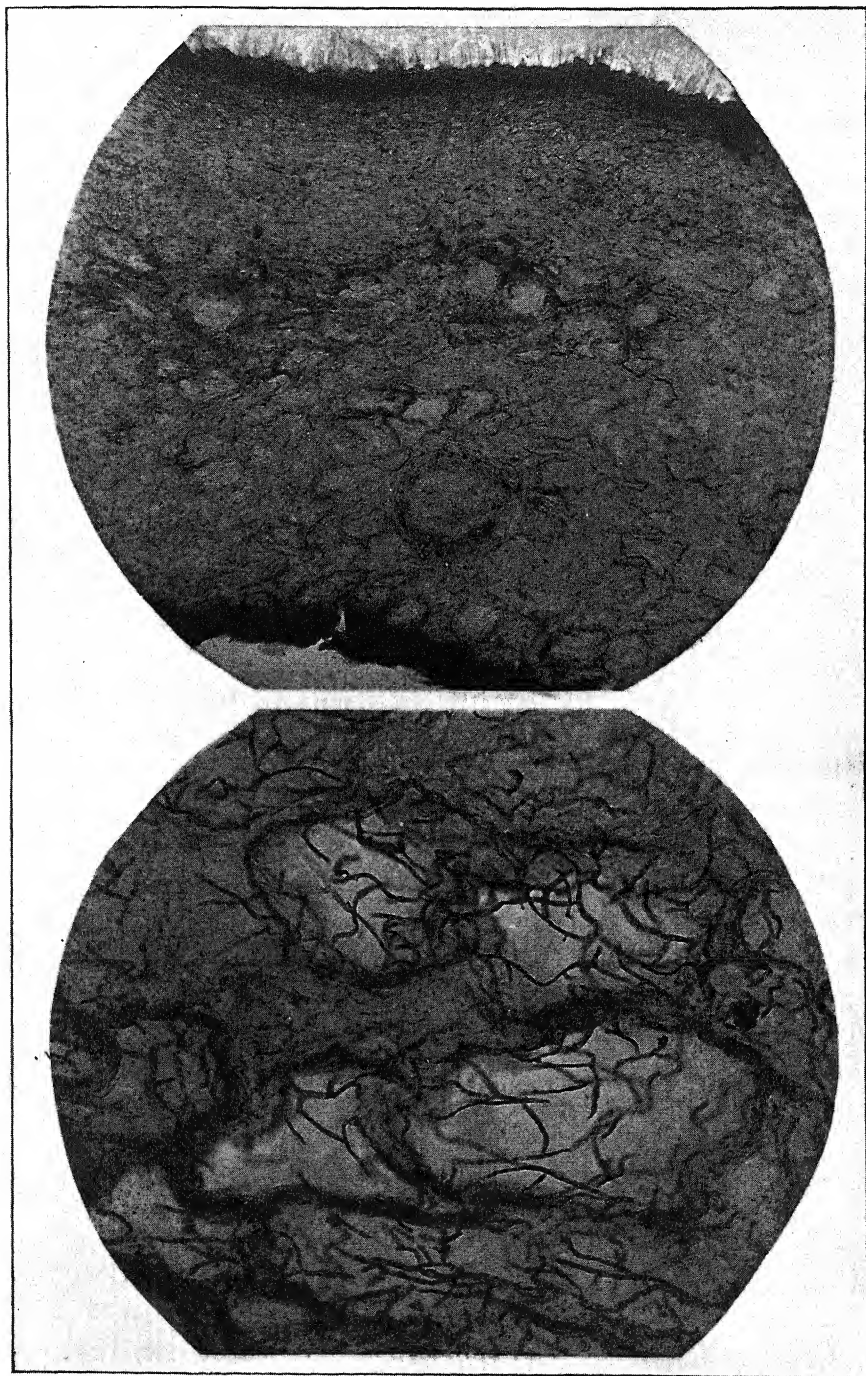
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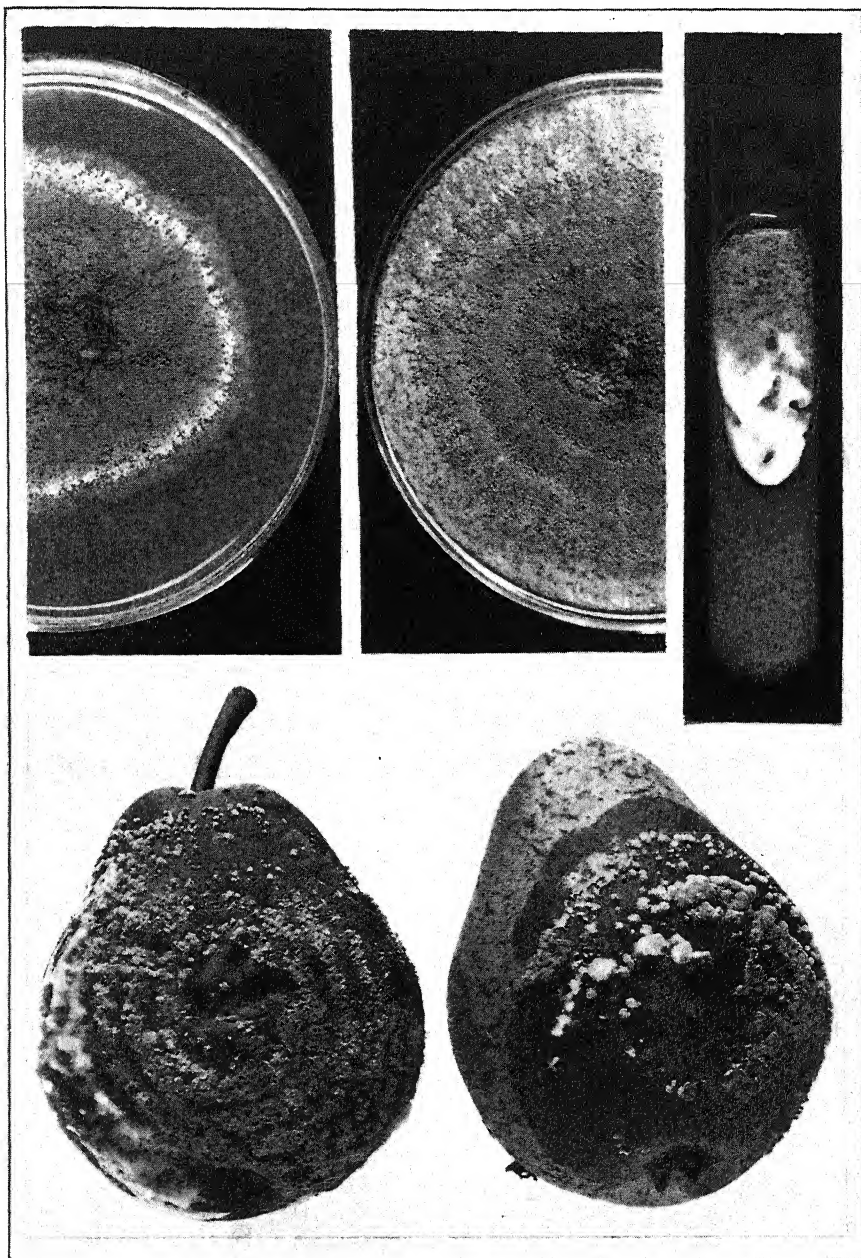
MONILINIA FRUCTICOLA





MONILINIA FRUCTICOLA





*MONILINIA FRUCTICOLA*





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## EXPLANATION OF PLATES

## PLATE 17

Apothecia of *Monilinia fructicola* (Wint.) Honey on mummied peach fruits. Upper, immature apothecial fundaments in various stages of development; lower, mature apothecia (natural size).

## PLATE 18

Photomicrograph of a free-hand cross-section (stained) through a portion of a pseudosclerotium of *Monilinia fructicola* (Wint.) Honey formed within the pericarp of cultivated peach fruit. Upper, showing above the black pseudoparenchymatous rind developed beneath the epidermis of the fruit upon which may be seen the typical trichomes; below the similar black pseudoparenchymatous rind which has separated the pseudosclerotium from the inner pulp of the fruit. Between these two outer coats lies the white fungous medulla intermixed with the brown dead collapsed host cells. Lower, a small portion of the above section showing in greater detail the host tissue and the fungous hyphae.

## PLATE 19

Conidial fructification of *Monilinia fructicola* (Wint.) Honey. Top left, 4-day-old petri dish culture; top middle, 8-day-old culture; top right, 4-day-old test-tube culture (all cultures on potato glucose agar held at 24° C.); bottom, natural infection on pear fruits. All artificial cultures are of strain H 441, isolated from ascospores from apothecia produced on peach mummies. (All figures about 4/5 natural size.)

## CONTRIBUTIONS TO OUR KNOWLEDGE OF WESTERN MONTANA FUNGI—II PHYCOMYCETES

PAUL W. GRAFF

The present contribution appears in continuation of previously published notes<sup>1</sup> on western Montana fungi. The earlier paper was confined to a consideration of the Myxomycetes gathered in that region. The present is restricted to the group of the Phycomycetes. It might well be called a "preliminary report" for there is no doubt that continued study devoted to these forms would reveal much of interest, and many more species than have been included here. The diversity of species listed, and the comparatively untouched condition of the field, is sufficient warrant for this assumption.

### CHYTRIDINEAE

#### FAMILY 1. OLPIDIACEAE

1. *OLPIDIUM PENDULUM* Zopf, in Schenk's Handb. 4: 555, *pl.* 66. 1890.

On pollen grains of *Pinus ponderosa* Dougl., Rattlesnake Creek, vicinity of Missoula, Missoula County, May 9, 1920, at about 4,000 feet elevation.

This species appeared attacking pollen grains gathered with an algal collection and kept in laboratory cultures. The parasitized pollen was in sufficient quantity for comparison, and to leave no doubt as to the identity of the fungus. As is usually the case with this species, the sporangia seen in the different grains were quite variable in both size and number. The zoöspores measured about  $5\mu$  in diameter, and were almost spherical in form. No resting sporangia were seen.

<sup>1</sup> Graff, Paul W., Contributions to our Knowledge of Western Montana Fungi—I. MYCOLOGIA 20: 101-113. 1928.

## FAMILY 2. WORONINACEAE

2. OLPIDIOPSIS SAPROLEGNIAE (A. Br.) A. Fisch. in Rabenh. Krypt.-Fl. 14: 38, fig. 4. 1892.

*Chytridium Saprolegniae* A. Br. Phys. Abh. Acad. Wiss. Berl. 1855: 61, pl. 5, fig. 23. 1855 (in part).

*Olpidiopsis Saprolegniae* (A. Br.) Cornu, Ann. Sci. Nat. Bot. V. 15: 145, pl. 3, fig. 1-9. 1872 (in part).

Growing upon *Saprolegnia monoica* Pringsh., from Yellow Bay Creek, University of Montana Biological Station grounds, east shore of Flathead Lake, July 8, 1918, at about 3,000 feet elevation:

In this collection the resting spores appeared typical, and very closely approximated in size those described by Fischer, measuring 65-70  $\mu$  in diameter, or 65  $\times$  75  $\mu$  when ellipsoidal in shape. The surface in this species is covered with coarse warting, and we find attached, in many cases, an "anhangszelle" of approximately half the diameter of the resting spore. *Chytridium Saprolegniae* of Braun has been found to be separable into two distinct species. In these we find the vegetative development somewhat confusing, but their resting spores offer no difficulties. In the case of *Olpidiopsis Saprolegniae* (A. Br.) A. Fisch., they vary from spherical to slightly ellipsoidal, and are strongly warted, while in *Pseudolpidium Saprolegniae* (A. Br.) A. Fisch. they are decidedly ellipsoidal, distinctly spinose, and somewhat larger. This latter species has thus far not been collected in Montana.

## FAMILY 3. RHIZIDIACEAE

3. RHIZOPHIDIUM AMPULLACEUM (A. Br.) A. Fisch. in Rabenh. Krypt.-Fl. 14: 101. 1892.

*Chytridium ampullaceum* A. Br. Phys. Abh. Acad. Wiss. Berl. 1855: 66-67, pl. 5, fig. 24-27. 1856.

Parasitic on *Oedogonium crenulato-costatum* Wittr., Yellow Bay Creek, between Yellow Bay and Bear Trap Mountains, Mission Range, Lake County, July 23, 1918, at about 4,500 feet elevation; on *Mougeotia* sp., marsh south of Finley Point, Flathead Lake, July 22, 1921, at about 3,000 feet elevation;

on *Oedogonium plagiosomum* Witttr., Rattlesnake Creek, above Greenough Park, vicinity of Missoula, September 26, 1925, at about 3,600 feet elevation.

The zoösporangia of this species are spherical, 7–8  $\mu$  in diameter, with a penetration tube through the host wall, but with no well-developed rhizoids. Discharge of zoöspores is through a cylindrical tube-like opening from the apex, similar to but slightly longer than that of *Phlyctochytrium equale* Atk., in fact the portion of that plant developed as sporangium outside the host appears very similar to *Rhizophidium ampullaceum* in both size and form. The tube in the case of *R. ampullaceum* is 4–5  $\mu$  long by 2  $\mu$  in diameter, while in *P. equale* it is of the same diameter but only 1.5  $\mu$  in length. The presence of this tube-like exit for the zoöspores forms a striking difference between this species and the other members of the genus reported here, most of which seem to have distinctive features in the manner of providing for spore escape.

Peterson (1910) says that this species should be transferred from the Chytridiineae to the Infusoria. He gives no reason for this conclusion, and without some convincing evidence to the contrary, the logical position for this fungus seems to be in the Rhizidiaceae.

4. RHIZOPHIDIUM GLOBOSUM (A. Br.) Schröt. Krypt.-Fl. Schles.  
3: 191. 1886.

*Chytridium globosum* A. Br. Phys. Abh. Acad. Wiss. Berl.  
1855: 34–39, pl. 2, fig. 14–20. 1856.

Parasitic on diatoms gathered in a surface towing among *Scirpus lacustris* L., in marsh south of Finley Point, Flathead Lake, July 22, 1921, at about 3,000 feet elevation.

The sporangia of this species are spherical, measuring 25–40  $\mu$  in diameter, and the largest of the several members of this genus reported here. The zoöspores escape through a large terminal opening which is not raised, and as a consequence the spherical form of the zoösporangium is maintained even after maturity and spore discharge. The rhizoids are quite reduced and similar in development to those of *Rhizophidium sphaerocarpum* (Zopf) A. Fisch.

5. RHIZOPHIDIUM POLLINIS (A. Br.) Zopf, Abh. Naturf. Ges. Halle 17: 79-107. 1887.

*Chytridium pollinis Pini* A. Br. Phys. Abh. Acad. Wiss. Berl. 1855: 40-41, pl. 3, fig. 1-15. 1856.

*Chytridium vagans* A. Br. Monats. Berl. Acad. 1856: 588. 1856.

On gymnospermous pollen gathered with an algal collection from Yellow Bay Creek, between Bear Trap and Yellow Bay Mountains, Mission Range, July 7, 1920, at about 3,600 feet elevation; on pollen of *Pseudotsuga mucronata* (Raf.) Sudw., gathered and placed in an algal culture from Yellow Bay Creek, University of Montana Biological Station grounds, east shore of Flathead Lake, July 11, 1921, at about 3,000 feet elevation.

Zoösporangia spherical to ovoid, 20-35  $\mu$  in diameter, with well-developed rhizoids ramifying through the host cell. One to several irregularly located openings are formed for the escape of the zoöspores instead of the usual single apical aperture. These openings are not raised or papillate, and are scarcely broader than the diameter of the zoöspores, being nearly round in form and measuring about 5  $\mu$  in diameter.

6. RHIZOPHIDIUM SPHAEROCARPUM (Zopf) A. Fisch. in Rabenh. Krypt.-Fl. 14: 95. 1892.

*Rhizidium sphaerocarpum* Zopf, Nova Acta Leop.-Carol. Akad. 47: 202, pl. 19, fig. 16-27. 1884.

Parasitic on *Mougeotia sphaerocarpa* Wolle, in a collection of various algae from Estey's Pond, vicinity of Bigfork, Lake County, July 7, 1921, at about 3,000 feet elevation; on *Mougeotia* sp., gathered in a swamp on Finley Point, east shore of Flathead Lake, July 22, 1921.

Sporangia ovoid, 12-16  $\times$  14-20  $\mu$ , becoming thistle-shaped with dehiscence through a single, proportionately large, circular, apical opening 4-6  $\mu$  in diameter, with either very much reduced rhizoids or contact with the interior of the host cell limited to merely a penetration tube without rhizoidal development. This species illustrates the intergradation of such forms as have been placed in the genus *Phlyctidium* and those similar in form but producing a rhizoidal development.

7. *Phlyctochytrium equale* Atk. Bot. Gaz. 48: 338, fig. 8. 1909.

On *Spirogyra Spreeiana* Rabenh., collected among other algae in a swamp on the south side of Finley Point, east shore of Flathead Lake, July 22, 1921, at about 3,000 feet elevation.

*Phlyctochytrium* is related to *Rhizophidium*, but differs from that genus in the development of a swollen or bulbous base of the penetration tube immediately within the wall of the host cell. The delicate rhizoids are produced from the lower portion of this subsporangial base. In this species both basal and exterior portion are of equal size, measuring  $5-7\ \mu$  in diameter. The zoöspores escape through a terminal opening which has the form of a short tube.

This opening is described by Atkinson (1909) as being round and having two small tooth-like projections on either side. These projections, shown in his figure of the species, are the side walls of the short tube. This cylindrical tube measures  $2\ \mu$  in diameter and  $1.5\ \mu$  in length.

As no partition wall is formed between the portions of this fungus outside and within the host, and as the content of the two portions appears to be used in the formation of zoöspores and is completely discharged at their maturity, it seems that we have here what is really a dumbbell-shaped sporangium neither wholly outside nor inside the host. This suggests that it is not improper to consider the members of this genus as representing intermediate forms between the genus *Rhizophidium* with its typically exterior sporangia and the genus *Entophlyctis*. In the latter genus the remnant of the fungus remaining on the exterior of the parasitized cell is seen as a papilla which serves later as a zoöspore exit, while the portion entering the host enlarges to form a sporangium. From the surface of this zoösporangium the rhizoids of the fungus are produced.

No such method of zoöspore escape as described by Petersen (1910) for *Phlyctochytrium stellatum* was suggested by the material of this collection which acted always in a manner typical for the genus.

## FAMILY 4. HARPOCHYTRIACEAE

Gobi (1899) is of the opinion that the genus *Harpochytrium* (*Fulminaria*) belongs among the stalked flagellates, while Wille (1900) places it among the algae, though believing it to be composed of reduced forms adapted to a saprophytic existence. In so doing he proposes the family Harpochytriaceae for its accommodation. Though standing more or less apart from the other genera, mycologists, as a rule, have disposed of this genus among the Rhizidiaceae. In this Gäumann (1926) concurs, but he considers it of totally independent origin from the other members of this family. Having very little in common with the Rhizidiaceae, it seems better that these forms be set apart, at least until some sufficient diagnostic character appears to entitle them to a place in a family already established. In so doing the use of Wille's name for the family is naturally retained, transferring it from the original position among the algae to one near the Rhizidiaceae. With this change the following emended diagnosis is suggested.

Plants nonchlorophyllaceous, fusoid, slightly curved or coiled, stalked or sessile, epiphytic, attached by a small disk-like basal expansion to the wall of the supporting plant, unicellular prior to maturity, later divided by a thin transverse wall into a sterile vegetative base and a zoösporangium. The zoöspores escape through the apex, after which the sterile base may grow upward filling the empty sporangium and the process of septation be repeated, successive sporangia being formed by proliferation much as in *Saprolegnia*. The zoöspores are uniflagellate, with the flagellum attached to the smaller forward end, somewhat irregular in shape, one side tending to be straight or slightly concave, hence swimming with a very erratic movement. The type species of the family is *Harpochytrium Hyalothecae* Lagerh.

8. HARPOCHYTRIUM HEDENII Wille, in Peterm. Mitteil. Erg. 371. 1900.

*Fulminaria Hedenii* Wille, Nyt. Mag. Naturvid. 41: 175. 1903.

*Rhabdium acutum* Dang. Ann. Myc. 1: 61-64, pl. 2. 1903.

On *Zygnema insigne* (Hass.) Kütz., Yellow Bay Creek, Uni-

versity of Montana Biological Station grounds, east shore of Flathead Lake, Lake County, July 11, 1921, at about 3,000 feet elevation; on *Zygnema* sp., Bitterroot River, vicinity of Missoula, Missoula County, May 23, 1925, at about 3,500 feet elevation.

Plants elongate, fusoid, slightly curved to one and a half times coiled, varying from  $5-6 \times 75-150 \mu$ , tending to taper more prominently toward the base than apex. The plant is attached by a small disk-like expansion within the wall of the host, but not penetrating completely to the interior of the cell; hence, seemingly not in contact with the cytoplasm. This disk serves as a holdfast, and from it no rhizoids develop. This characteristic makes the fungus, to all appearances, an epiphyte rather than a parasite. The Montana material is thus in full agreement with Atkinson's (1903) description of this species. On the other hand, Dangeard (1903) considers that the fungus penetrates to the interior of the host cell, and develops the disk in contact with the cytoplasm. Lagerheim (1890) suggests that there are probably rhizoids extending from the disk-like base into the host cell. This has never been substantiated by other observers, and is no more than surmise on his part.

A further point of disagreement is connected with sporangial development. As the plant proceeds toward maturity the greater portion of the cytoplasm, variable in amount and constituting anywhere from three-fourths to five-sixths of the upper part, assumes a more dense appearance than the remainder. While the zoöspores are being formed in this upper portion, the lower part is cut off by a thin wall, thus creating at maturity a vegetative and a reproductive portion from what up to that time showed only a unicellular structure and the presence of vegetative activity. After the escape of the zoöspores from the apex, the lower sterile portion of the plant may grow upward within the empty sporangium till nearly filling it, when the process of separation into zoösporangium and sterile base is repeated. This proliferation frequently takes place two or three times. Here again we are in agreement with Atkinson as well as, in this instance, with Lagerheim.

Dangeard claims that after the complete emptying of the sporangium, formed from the entire plant, a new sporangium



may be developed within the old, suggesting a possibility of rejuvenation. Obviously this is an impossibility unless growth is provided for by a remnant of cytoplasm or by the method Gobi suggests. Gobi (1899) claims for *Harpochytrium Hyalothecae* Lagerh. (*Fulminaria mucophila* Gobi), a closely related species, that there is no sterile portion remaining after the zoöspores have escaped, but that if a zoöspore remain behind it may germinate there and develop a new sporangium, or plant, within the old, and so give the appearance of proliferation. Due to the epiphytic, rather than parasitic, nature of the members of this genus it is obviously not impossible for a chance zoöspore to develop in this manner. The condition is unusual, however, and Gobi seems to be the only one to report its occurrence. It is also not inconceivable that at times the entire content of the cell might be transformed into zoöspores. In this event no further growth could take place and no secondary zoösporangia could be formed, unless some such occurrence as Gobi mentions should take place. At the end of the growth period for a plant, after the last proliferation has taken place, either the entire cytoplasm is used in the development of zoöspores or the sterile base dies and growth ceases. Following this a chance zoöspore might easily remain in the lower portion of the otherwise empty sporangium, develop there, and produce a new plant within the old. Gobi's observations are neither impossible nor illogical so far as they go. The trouble is that he did not follow the plants through as extended or as careful a series of observations as he should. A sterile basal area has been described as characteristic of *H. Hyalothecae* Lagerh., *H. Hedenii* Wille, and *H. intermedium* Atk., the three recognized species of the genus, and so for the entire membership of the family.

#### FAMILY 5. RHIZOPHLYCTACEAE

9. POLYPHAGUS EUGLENÆ (A. Br.) Nowak. in Cohn, Beitr. Biol. Pflanz. 2: 203, pl. 8-9. 1876.

*Chytridium Euglenae* A. Br. Phys. Abh. Acad. Wiss. Berl. 1855: 46. 1856.

Parasitic on *Euglena viridis* Ehrenb., vicinity of St. Ignatius, Lake County, June 28, 1920, at about 3,000 feet elevation; same host and locality, June 25, 1921.

*Euglena viridis* Ehrenb. is especially plentiful in the numerous glacial pot holes of the region immediately north of the town of St. Ignatius. There is a recurrence each season, at approximately the time these collections were made, of a sufficient number of the organisms to form a complete scum over the surface of many of these ponds. The water is warm and, with evaporation taking place, a considerable deposit of *Euglena* in the encysted condition is left about the muddy margin. *Polyphagus* was not uncommon among the floating material, and its recurrence under the prevailing conditions may be expected. In fact at the time of the collections cited above, the attack by this fungus might be considered of epidemic proportions.

10. SPOROPHYCTIS ROSTRATA Serb. Scripta Bot. Hort. Univ. Imp. Petrop. 24: 164-165, *pl.* 1-2, *fig.* 13-35. 1907.

On *Draparnaldia plumosa* (Vauch.) Agardh, Yellow Bay Creek, between Yellow Bay and Bear Trap Mountains, Mission Range, July 23, 1918, at about 4,000 feet elevation.

This very interesting little fungus seems to have been reported heretofore only by Serbinow in the description of his original collection made in the vicinity of St. Petersburg. In the Montana material akinet formation was observed essentially as described by Serbinow, but conjugation between individuals and developments depending upon it were not seen. Though a number of other collections were made in the same locality with the hope that the various stages of development might be reviewed, the parasite only appeared in the one.

#### FAMILY 6. CLADOCHYTRIACEAE

11. *Cladochytrium maculare* (Wallr.) comb. nov.

*Physoderma maculare* Wallr. Fl. Crypt. Germ. 2: 192. 1833.

*Protomyces macularis* (Wallr.) Sacc. Michelia 1: 13. 1879, non Fuckel.

*Cladochytrium Alismatis* Büsgen, in Cohn, Beitr. Biol. 4: 280. 1887.

On *Alisma brevipes* Greene, collected in a swamp on the south side of Finley Point, east shore of Flathead Lake, July 22, 1921.

The present collection records a new host as well as a new locality for this fungus. Previously the species has only been reported as attacking *Alisma Plantago* L., in Europe and the eastern United States, and *Alisma subcordata* Raf., in Manitoba. *Protomyces macularis* of Fuckel (1869) seems to be synonymous with *Doassansia Alismatis* Cornu. There is no doubt, however, that *Physoderma maculare* Wallr. is the *Cladochytrium Alismatis* of Büsgen, and of this collection. Wallroth's original specimens were examined and well figured by DeBary (1864). Büsgen in making the transfer from *Physoderma* to *Cladochytrium* changed the specific name as well, apparently with no other reason or intent than to have the specific name conform with that of the host. This change has been perpetuated by all later workers who have had occasion to cite this species. Adherence to the rules of priority makes necessary the use of Wallroth's rather than Büsgen's specific name.

12. UROPHLYCTIS ALFALFAE (Lagerh.) Magn. Ber. Deuts. Bot. Ges. 20: 291-296, *pl.* 15. 1902.

*Cladochytrium Alfalfae* Lagerh. Bull. Herb. Boissier 3: 53-74. 1895.

Causing crownwart of cultivated *Medicago* sp., vicinity of Polson, Lake County, July 15, 1921.

Though not uncommon to the westward this seems to be the first collection of this parasite reported from Montana. The disease has been considered common only west of the Sierra Nevada and Cascade Mountains. O'Gara has reported the trouble in the Salt Lake Valley of Utah, and McCallum has reported it as present in Arizona, thus carrying the range eastward in the south and central west. The present collection extends the range of the disease eastward from its more northern habitat in Oregon and Washington, though still failing to carry it beyond the Rockies in this section.

13. UROPHLYCTIS PLURIANNULATUS (Berk. & Curt.) Farlow, Rhodora 10: 12-13. 1908.

*Uromyces pluriannulatus* Berk. & Curt. Grevillea 3: 57. 1874.

*Uromyces hemisphaerica* Speg. Anal. Soc. Ci. Argent. 12: 26. 1881.

*Urophlyctis Kriegeriana* Magn. Sitzber. Ges. Naturf. Freu. Berl. 1888: 100. 1888.

*Urophlyctis hemisphaerica* (Speg.) Syd. Ann. Myc. 1: 517-518. 1903.

On leaves of young plants of *Osmorrhiza longistylis* (Torr.) DC., vicinity of Yellow Bay, Flathead Lake, July 10, 1920.

This seems to be a new host as well as a new locality for this fungus. Both leaf blades and their petioles were found to be attacked. Small pustules are formed, the surface of which becomes granular in appearance and of a yellow color. With age this color changes to brownish. The effect on the host simulates much the appearance of a rust, and accounts for the errors in the earlier determinations. Farlow (1908) recognized the mistake of Berkeley and Curtis, and also the fact that *Urophlyctis Kriegeriana* Magn. is synonymous with this species. Sydow (1903) recognized the identity of *U. Kriegeriana* with Spegazzini's species of *Uromyces*, but failed to note these as the same fungus Berkeley and Curtis had described at an earlier date.

## OOMYCETINEAE

### FAMILY 7. LAGENIDIACEAE

14. MYZOCYTIIUM PROLIFERUM Schenk, Contr. Zellen 10. 1858.

*Pythium proliferum* Schenk, Verh. Phys.-Med. Ges. Wurzb. 9: 20. 1857, non DeBary.

*Lagenidium globosum* Linds. Synop. Saproleg. 54. 1872.

Parasitic in cells of *Cladophora Kuetzingiana* Grunow, Rattlesnake Creek, Greenough Park, Missoula, September 23, 1919; in *Zygnema cruciatum* (Vauch.) Agardh, marsh south of Finley Point, Flathead Lake, July 22, 1921.

Vegetatively this plant forms a rudimentary hypha or pseudo-hypha of irregularly arranged somewhat ovoid or ellipsoidal cells. These are transformed later into zoösporangia within which the biflagellate zoöspores are developed; or adjacent cells may conjugate and a resting spore be formed in one of each conjugating pair. In the closely related *Lagenidium* we find the antheridial cell much smaller than that in which the oöspore is developed. *Myzocyttium* does not show as much differentiation. The

antheridial and oögonial cells are, superficially at least, indistinguishable from one another till the formation of the oöspore presents a distinguishing mark.

15. *LAGENIDIUM RABENHORSTII* Zopf, Verhandl. Bot. Vereins. Prov. Branden. 20: 77. 1878.

Attacking vegetative cells of *Spirogyra orthospira* Nag., in an algal collection from Estey's Pond, vicinity of Bigfork, Lake County, July 1, 1921; within cells of *Oedogonium plusiosporum* Wittr., in a collection of miscellaneous algae, marsh south of Finley Point, Flathead Lake, July 22, 1921.

The antheridia of this species are formed as short cells cut off in the vicinity of the irregular, long, swollen oögonial cells with which they conjugate by means of a short penetration tube. The oöspores are spherical, measuring 15–20  $\mu$  in diameter.

#### FAMILY 8. BLASTOCLADIACEAE

16. *BLASTOCLADIA RAMOSA* Thaxter, Bot. Gaz. 21: 45–52, pl. 3, fig. 1–16. 1896.

On submerged sticks of *Populus trichocarpa* T. & G., shore of Yellow Bay, Flathead Lake, July 12, 1919; on submerged sticks of *Betula fontinalis* Sarg., in a quiet pool of Yellow Bay Creek, east shore of Flathead Lake, July 1, 1921, at about 4,500 feet elevation.

From the rhizoidal base the plant develops a single stalk of uniform diameter, and of about half the height of the plant. From this stalk numerous branches develop in a somewhat irregular subdichotomous manner. The branched head of the plant has a breadth of from 250 to 550  $\mu$ ; very rarely this is greater and may reach a diameter of 650  $\mu$ . On the ends of the branches, or slightly below, zoösporangia or conidia are developed, both of nearly the same size and shape. The conidia are broadly ovoid, 11–15  $\times$  30–32  $\mu$ , rounded at the apex and truncate at the base. The zoösporangia are likewise broadly ovoid, 12–16  $\times$  30–32  $\mu$ , bluntly pointed or broadly papillate at the apex and truncate at the base. The conidia possibly partake more of the nature of resting spores though their walls are not heavy, being in fact but very slightly thicker than those of the zoösporangia.

## FAMILY 9. LEPTOMITACEAE

17. SAPROMYCES REINSCHII (Schröter) Fritsch, Österr. Bot. Zeits. 43: 420. 1893.

*Naegeliella Reinschii* Schröter, in Engler & Prantl, Nat. Pfl. 1<sup>1</sup>: 103, fig. 85. 1897.

*Rhipidium elongatum* Cornu, Ann. Sci. Nat. Bot. V. 15: 15. 1871.

On submerged twigs of *Pseudotsuga mucronata* (Raf.) Sudw., Yellow Bay, east shore of Flathead Lake, July 12, 1919.

The plant seems to be attached to the substratum directly by its lower segment which may become somewhat distorted but does not produce a definite rhizoidal development. The vegetative portion is characteristically constricted with some regularity, and has the appearance of segmentation which in reality may or may not occur. Branching is profuse, and the plant spreading. Either zoösporangia, oögonia, or antheridia may be produced at the upper ends of the segments, whether terminal or not, and most often in whorls of from two to five. At times zoösporangia and oögonia or zoösporangia and antheridia are found associated, though in no case were oögonia and antheridia seen to be developed in the same whorl.

## FAMILY 10. SAPROLEGNIACEAE

18. SAPROLEGNIA DICLINA Humph. Trans. Am. Phil. Soc. 17: 109, pl. 17, fig. 50-53. 1892.

*Saprolegnia dioica* DeBary, Bot. Zeit. 46: 619, pl. 10, fig. 12-13. 1888, non Pringsh., non Schröter.

On fly larvae placed in a mixed algal culture from Yellow Bay Creek, University of Montana Biological Station grounds, east shore of Flathead Lake, Lake County, July 1, 1921.

Oögonia, while not numerous, were produced in the culture. These varied from 50-75  $\mu$  in diameter, and had from 7-16 egg cells developed in each. Antheridia were in evidence wherever oögonia were present. In this association they were numerous and almost completely covered the oögonia, in which they form a conspicuous characteristic for this species.

19. SAPROLEGNIA MONOICA Pringsh. Jahrb. Wiss. Bot. 1: 292, pl. 19-20. 1858.

*Achlya intermedia* Bail, 35te Versamml. Deuts. Naturf. Aerzte Konigs. 1860: 5. 1861.

*Diplanes saprolegnioides* Leitg. Jahrb. Wiss. Bot. 7: 374, pl. 24. 1869.

On flies in a culture of *Vaucheria sessilis* (Vauch.) DC., kept in the laboratory, from Yellow Bay Creek, University of Montana Biological Station grounds, east shore of Flathead Lake, July 8, 1918; on flies placed in a mixed algal culture dredged from Yellow Bay, Flathead Lake, July 16, 1921.

This species has been reported previously by Atkinson (1897) from Alabama, and by Pieters (1915) from Michigan. It is evidently of much more frequent occurrence in Europe than in the United States. The Montana material corresponds closely with the characteristics of the species rather than with either var. *glomerata* Ties., or var. *vexans* Pieters, which have been reported as more common in this country. The wide separation of the three habitats for the species would seem to indicate the possibility that further study will give more prominence to this form of the fungus than it has formerly received. In the Montana material the oögonia were in the usual racemose form, and associated with these were antheridial branches of the androgynous type. Oöspores were found to number from 5-14 in an oögonium, rarely more, and measured 16-20  $\mu$  in diameter. Zoösporangia were slender, cylindrical to clavate, and frequently found proliferating.

20. SAPROLEGNIA PARASITICA Coker, The Saprolegniaceae, 57-59, pl. 18, fig. 1-12. 1923.

From a diseased spot on the side of a large fish of the sucker variety caught in Yellow Bay, Flathead Lake, July 28, 1921.

The fish, weighing about three pounds, was so sluggish in its actions that it was easily snagged with a bare hook and brought to the surface. The diseased spot on its side was somewhat over an inch in diameter, discolored, somewhat darker than normal in the center with a lighter margin. Macroscopically the disease appeared to penetrate for about half an inch, though undoubtedly actual penetration into the flesh was much deeper.

21. ISOACHLYA MONILIFERA (DeBary) Kauff. Am. Jour. Bot. 8: 231. 1921.

*Saprolegnia monilifera* DeBary, Bot. Zeit. 46: 629-631, pl. 9, fig. 6. 1888.

On flies placed in a mixed algal culture from Rattlesnake Creek, Greenough Park, Missoula, September 26, 1925; on flies in culture jar with *Spirogyra* sp., from Bitterroot River, vicinity of Lolo, Missoula County, May 12, 1926, at about 3,500 feet elevation.

Oögonia appeared very abundantly, in chains, individuals varying considerably in size, 45-80  $\mu$  in diameter, but with the greater portion about 60  $\mu$ . No antheridia were seen. Zoösporangia were relatively few in number, moderately long, with their greatest breadth well toward the tip. Growth was quite profuse.

22. ACHLYA AMERICANA Humph. Trans. Am. Phil. Soc. 17: 116, pl. 14, fig. 7, 9, 10. 1892 [1893].

On submerged decaying sticks of *Populus trichocarpa* T. & G., Yellow Bay, east shore of Flathead Lake, July 12, 1919; on submerged sticks of *Alnus tenuifolia* Nutt., in warm slough from the Bitterroot River, near Ft. Missoula, vicinity of Missoula, May 17, 1925.

This species seems to approach very closely to *Achlya DeBaryana* Humph., from which it differs in having short-stalked oögonia with many more pores in their walls and smaller oöspores. Petersen (1910) considers this a variety of what he calls *A. polyandra* (Hildebr.) DeBary, as in his opinion *A. polyandra* Hildebr., and *A. polyandra* DeBary, are synonymous. In this combination Petersen fails to find support. *A. polyandra* DeBary is *A. DeBaryana* Humph., DeBary's name being antedated by that of Hildebrand, while *A. polyandra* Hildebr. is recognized as a distinct species known to DeBary and named by him *A. gracilipes*. The former of these is not recognized as an American species. The latter has been found occasionally in this country. If *A. americana* Humph. is to be considered as a variety, it should be not as Petersen suggests but as *Achlya DeBaryana* Humph., var. *americana* (Humph.).



23. *ACHLYA RACEMOSA* Hildebr. Jahrb. Wiss. Bot. 6: 249-269, pl. 15-16. 1867.

On flies in a mixed algal culture from Yellow Bay Creek, Biological Station grounds, east shore of Flathead Lake, July 17, 1918; on flies in a culture of *Zygnema* sp., from Estey's Pond, vicinity of Bigfork, Lake County, July 26, 1919; on flies placed in a culture of *Spirogyra orthospira* Nag., from Estey's Pond, July 1, 1921.

In this material the oögonia were formed on short lateral branches. They showed a variation of from 45-70  $\mu$  in diameter, with 60  $\mu$  the more usual size. Antheridial branches arise from a position on the oögonial stalks immediately below the oögonia. There are usually but one or two of these antheridia to each oögonium, though occasionally there are more.

#### FAMILY 11. PERONOSPORACEAE

24. *ALBUGO BLITI* (Biv.-Berhn.) O. Kuntze, in Magn. Abh. Natur. Ges. Nürnberg. 11: 53. 1897 [1898].

*Uredo Bliti* Biv.-Bernh. Stirp. Sicula Manip. 3: 11. 1815.

*Cystopus Bliti* Lévl. Ann. Sci. Nat. III. 8: 371. 1847.

On *Amaranthus retroflexus* L., vicinity of Polson, Lake County, July 8, 1919; on the same host, vicinity of Bigfork, Lake County, July 17, 1920.

25. *PERONOSPORA EFFUSA* (Grev.) Rabenh. Herb. Myc. 1: 264. 1880.

*Botrytis effusa* Grev. Fl. Edin. 468. 1824.

*Botrytis farinosa* Fr. Syst. Myc. 3: 404. 1829.

On leaves of *Chenopodium album* L., near road east shore of Flathead Lake, between the Biological Station and Bigfork, Lake County, July 7, 1919.

Conidiophores were hypophyllous, on the lower leaves of the host, causing them to turn yellowish at first, later brown, after which they ultimately dry, wither, and fall.

26. PERONOSPORA PARASITICA (Pers.) DeBary, Ann. Sci. Nat. Bot. IV. 20: 110. 1863.

*Botrytis parasitica* Pers. Obs. Myc. 1: 96, pl. 5, fig. 6. 1796.

On the lower leaves of *Cogswellia montana* (Coul. & Rose) Jones, Hell Gate Canyon, vicinity of Missoula, May 12, 1925; on *Cogswellia leptocarpa* (Nutt.) Jones, vicinity of Missoula, May 15, 1926; on *Cogswellia montana* (Coul. & Rose) Jones, Ft. Missoula, vicinity of Missoula, May 18, 1926.

The various species of *Cogswellia* are very generally infected with this disease in this region, and are consequently of importance as carriers of the fungus.

27. PERONOSPORA TRIFOLIORUM DeBary, Ann. Sci. Nat. Bot. IV. 20: 117. 1863.

On leaves of cultivated *Medicago sativa* L., vicinity of Missoula, June 3, 1926; on same host, vicinity of Lolo, Missoula County, June 7, 1926.

So far as I am aware, this disease of our cultivated Alfalfa has been reported from Colorado, but not farther north in the Rocky Mountain region. The conidiophores were wholly confined to the under side of the leaflets which, as a consequence of the disease, showed a decided tendency to curl downward. The conidia measured  $18-20 \times 21-27 \mu$ , not as wide a range in size as usually found, and not as large as the figures given by Stewart (1908) for the fungus as found in New York state. This New York material, however, according to Stewart, produces spores of greater dimension than the typical. Oöspores were not seen. This species is a very close relative of *Peronospora Viciae* Berk., from which it differs chiefly in the branching of the conidiophores. These branches form broad obtuse angles in *P. Trifoliorum* and acute angles in *P. Viciae*. The spores developed in either case are of the same size.

28. BASIDIOPHORA KELLERMANII (Ellis & Hal.) Wilson, Bull. Torrey Club 34: 394-395. 1907.

*Peronospora Kellermanii* Ellis & Hal. in Ellis & Everh. N. Am. Fungi 2201. 1889.

On *Iva xanthiifolia* Nutt., vicinity of Polson, Lake County,

July 8, 1919; also vicinity of Stevensville, Rivalli County, July 14, 1925, at about 4,000 feet elevation.

29. PLASMOPARA GERANII (Peck) Berl. & DeToni, in Sacc. Syll. Fung. 7: 242. 1888.

*Peronospora Geranii* Peck, Rept. N. Y. State Mus. 28: 63. 1874.

On leaves of *Geranium viscosissimum* F. & M., vicinity of Yellow Bay, east shore of Flathead Lake, July 14, 1920; on leaves of *Erodium cicutarium* (L.) L'Her., on the bank of Rattlesnake Creek, Greenough Park, Missoula, May 23, 1923.

30. PLASMOPARA OBUDCENS (Schröt.) Schröt. in Cohn, Krypt.-Fl. Schles. 1<sup>3</sup>: 238. 1889.

*Peronospora obducens* Schröt. Hedwigia 16: 129. 1877.

On *Impatiens aurella* Rydb., vicinity of Yellow Bay, east shore of Flathead Lake, July 6, 1921; on *Impatiens* sp., Lolo Creek, Missoula County, June 24, 1925, at about 4,200 feet elevation.

31. PLASMOPARA RIBICOLA Schröt. in Cohn, Krypt.-Fl. Schles. 1<sup>3</sup>: 238. 1889.

*Peronospora ribicola* Schröt. Jahr. Schles. Gesell. 1883: 179. 1883.

On wild gooseberry, Spring Gulch, Rattlesnake Valley, vicinity of Missoula, June 14, 1920, at about 3,600 feet elevation; on young plants of *Ribes* sp., on south side of Mt. Sentinel, Pattee Canyon, vicinity of Missoula, May 29, 1923, at about 4,500 feet elevation.

32. BREMIA LACTUCAE Regel. Bot. Zeit. 1: 666, pl. 3. 1843.

*Botrytis ganglioniformis* Berk. Jour. Hort. Soc. Lond. 1: 51. 1846.

*Peronospora gangliiformis* DeBary, Ann. Sci. Nat. Bot. IV. 20: 108. 1863.

On leaves of *Lactuca virosa* L., vicinity of Yellow Bay, east shore of Flathead Lake, July 3, 1918; on the lower leaves of *Lactuca Ludoviciana* (Nutt.) DC., vicinity of Polson, Lake County, July 12, 1921.

## ZYGOMYCETINEAE

## FAMILY 12. MUCORACEAE

33. ABSIDIA CAERULEA Bain, Bull. Soc. Bot. 1: 184. 1889.

Appearing on bread in a laboratory culture, Missoula, February 19, 1918; also February 26, 1923.

34. MUCOR ABUNDANS Povah, Bull. Torrey Club 44: 292-293, pl. 17, fig. 1-6. 1917.

On bread in a laboratory culture, Missoula, February 23, 1922; also February 20, 1925.

This species shows in its general appearance considerable resemblance to *Mucor hiemalis* Wehm., but differs in being decidedly racemose rather than usually unbranched, and in its pyriform instead of spherical to oval columella. The spores in both cases are nearly of a size, but differ in shape. In the case of *M. abundans* they are globose to subellipsoidal and measure  $3-6 \times 4-8 \mu$ , most often globose and  $3-5 \mu$  in diameter. In *M. hiemalis* they are ellipsoidal or somewhat irregularly so, measuring  $2-5.5 \times 3-8.5 \mu$ , with a usual size of  $3 \times 7 \mu$ .

35. MUCOR CIRCINELLOIDES Van Tiegh. Ann. Sci. Nat. Bot. VI. 1: 94. 1875.

On a bread culture, Missoula, February 19, 1918; also February 26, 1923, and February 20, 1925.

36. MUCOR MUCEDO (L.) Bref. Bot. Unters. 1: 7, pl. 1-2. 1872.

*Mucor Mucedo* L. Sp. Pl. 2: 1655. 1753, in part.

*Mucor sphaerocephalus* Bull. Hist. Champ. Fr. 112, pl. 480, fig. 2. 1791-98.

On bread culture, Missoula, February 19, 1918; also February 23, 1922, and March 2, 1923.

37. MUCOR PLUMBEUS Bonord. Abh. Naturf. Ges. Halle 8: 109. 1864.

*Mucor spinosus* Van Tiegh. Ann. Sci. Nat. Bot. VI. 4: 390. 1876.

On bread culture, Missoula, February 23, 1922, and February 20, 1925.

In this species we find a characteristic spinose development on the upper portion of the columella. *Mucor spinescens* Lend.

is undoubtedly a close relative if not a mere variation of the above. Their chief characteristics are decidedly similar though in the latter species the sporangiophores are reported as much shorter, 1 mm. instead of 1 cm., and the sporangia 60–68  $\mu$  instead of 100  $\mu$  in diameter. The spores in both cases are found to be spherical, and measure 5–8  $\mu$  in diameter. The case of *M. plumbeus* and *M. spinescens* is only one among the number to be found in the Mucoraceae where a critical study is needed to determine the identity or validity of species.

38. *MUCOR RACEMOSUS* Fres. Beitr. Mycol. 1: 12, pl. 1, fig. 24–35. 1850.

*Pleurocystis Fresenii* Bonor. Allgem. Mycol. 124. 1851.

On bread culture, Missoula, February 19, 1918; also March 2, 1923, and February 20, 1925.

39. *RHIZOPUS NIGRICANS* Ehrenb. Nova Acta Leop.-Carol. Akad. 10: 198, pl. 11, fig. 1–7. 1820.

*Mucor stolomifer* Ehrenb. Sylvae Myc. Berol. 13: 25. 1818.

On bread culture, Missoula, February 19, 1918; also February 23, 1922, and February 26, 1923.

40. *THAMNIDIUM ELEGANS* Link, Observat. 45, pl. 2, fig. 45. 1809.

*Mucor elegans* Fries, Syst. Myc. 3: 322. 1829.

*Ascophora elegans* Corda, Icon. Fung. 3: 14, pl. 2, fig. 43. 1839.

*Thamnidium elegans* Van Tiegh. Ann. Sci. Nat. Bot. V. 17: 321, pl. 23, fig. 57–60. 1873.

On bread culture, Missoula, February 23, 1922; also February 20, 1925.

#### FAMILY 13. PIPTOCEPHALIDACEAE

41. *PIPTOCEPHALIS FRESENIANA* DeBary, Abh. Senckenb. Naturf. Ges. 5: 356, pl. 43, fig. 17–19. 1865.

Parasitic on *Mucor circinelloides* Van Tiegh., Missoula, February 26, 1923; also on *Mucor racemosus* Fres., Missoula, March 2, 1923, and February 20, 1925, all from bread cultures.

## FAMILY 14. CHAETOCLADIACEAE

42. CHAETOCLADIUM JONESII (Berk. & Curt.) Fres. Beitr. Myc. 97, *pl.* 12, *fig.* 5-12. 1863.

*Botrytis Jonesii* Berk. & Curt. Ann. Mag. Nat. Hist. XIII. 2: 760, *pl.* 15, *fig.* 12. 1854.

In bread cultures, parasitic on *Mucor abundans* Povah, and *Mucor Mucedo* (L.) Bref., Missoula, February 23, 1922.

## FAMILY 15. ENTOMOPHTHORACEAE

43. EMPUSA MUSCAE Cohn, Nova Acta Leop.-Carol. Akad. 25: 301-360, *pl.* 9-11. 1855.

*Myiophyton Cohnii* Leb. in Denk. Schw. Ges. Naturw. 15: 26. 1857.

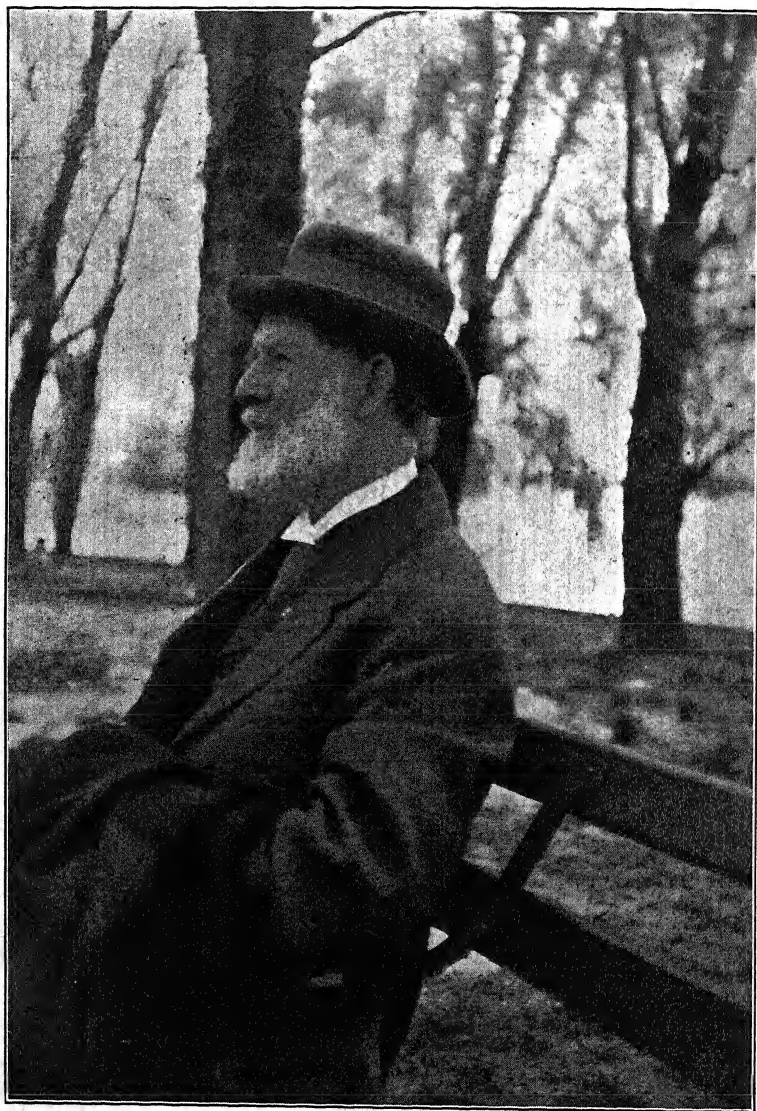
On dead flies, University of Montana Biological Station, Yellow Bay, July 2, 1921; on dead house flies, Missoula, August 29, 1925.

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ERWIN F. SMITH

FREDERICK V. RAND

(WITH PLATE 20)

Dr. Smith's span of life covers a period of swiftly changing ideas and rapidly mounting achievements in the history of plant pathology in America. He saw its birth as a science; he, himself, contributed more than any other one man to its mature development; and, what is rarely experienced by creative genius, he lived to see it secure in its position among the sister sciences. Surely an enviable record!

At the time of their early studies of bacteria in relation to human and animal disease, Pasteur and Koch found the state of knowledge sadly chaotic and conflicting. At the same level Smith encountered the problem of bacterial disease in the realm of plant life. In those first years he fought the battle almost single-handed, but the foundations which he laid back of these early steps were so sound and secure that they have withstood the tread of many later generations.

My own association with Dr. Smith began at a time just succeeding the publication (1907) of his first memorable contribution on plant cancers of bacterial origin. The introduction to him well illustrated his characteristic human interest in everybody, and especially in those who were earnestly engaged in any honest endeavor. It had fallen to him to grade my Civil Service examination paper, without of course knowing to whom it belonged. Some weeks later he chanced to see a slip of paper in my handwriting and, noticing at once the similarity, asked that

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the writer be found and brought to him. I shall never forget that first meeting—the light in his eye, known to his friends but never shown in his photographs, the kindly interest and advice, the sincere invitation to come again and again—a meeting which led the way to long years of association, through which I learned to honor him as one of the great men of science and to love him as a father.

Toward the group of workers immediately under his charge he was almost patriarchal in attitude; there was always the close relationship on the human side. He loved to have the whole group at his home; we were often there and always welcome—at Christmas, on Thanksgiving Day, and on many other occasions. After the meal he often exchanged reminiscences or read to us gems of his own more recent sonnets. We had all read his book of sonnets and translations published in 1915 and we knew that another volume was on the way. He was expert in pencil drawings and the woodcuts in this first book of sonnets were by his own hand. His pencil drawings were made with extreme care and, with his eye trained to note details instantly, were useful adjuncts to his scientific work, especially in the earlier years.

He was always giving a helping hand to somebody; his generosity was nothing short of phenomenal. Today it might be a needy fellow scientist in Austria; tomorrow, an injured watchman in the building where he worked. Any youngster with a whit of sense and ambition was sure of an audience with him, and many a struggling youth he helped through school. To any worthy person or object he was ready to give of himself and of his means.

Toward woman's suffrage he lent his support from the first. He always believed that women should be given a fair chance in science, and this at a time when such opportunities were rare outside the teaching profession.

However reticent he might be toward outsiders about giving out professional secrets prior to publication, he was always freely communicative to members of his own staff, and not only with results already obtained but also regarding his ideas, plans, and hypotheses for future investigation.

As to essentials, he was one of the most modest of men, often to the point of shyness or even discomfort; yet in very minor points of detail he was sometimes quite exacting as to his due. On expressing my deep appreciation at the opportunity of becoming one of his group of workers he said: "I am afraid you will be disappointed for I am not as big a man as you think I am." More recently, when one of the members of his staff had urged him strongly to write an autobiography, he replied: "There is nothing in my life to interest anybody." And these statements were made in all sincerity and with no least hint of affectation.

His intense love of art in all its forms—music; literature; poetry; painting; sculpture; vases, of which he collected many—was well known to all his friends. Anything in brilliant colors made to him a great appeal. He loved the flaming reds of canna and scarlet salvia; and yet he possessed also a keen sense for slight differences in color.

In later years the terraced garden on the steep hillside back of his house was a source of keen delight and pride to him. Walking and sawing cord wood were his favorite forms of exercise.

He loved children, dogs, and jokes, especially those on the Scotch. Next door, two children who had lost their father found in him a true friend. He loved to talk with them and tell them stories, and many a book was received by them from his hand. Just before his marriage in 1914, one of these children was heard to remark: "I wonder if it will be the same as it was or if it will be as if he had never been."

His interests were as wide as the world and wider. One day he went into a Boston bookstore and asked to be shown all the books on Italy. Before leaving he had purchased thirty! On another occasion he bought copies of all the periodicals sold in the bookstalls along the Seine in order to find out what was being offered to the Paris public. He took the London Times and the Manchester Guardian. More recently, he subscribed to the Congregationalist, having read an article and editorial on evolution following the Bryan controversy—"For," he said, "any religious journal that is open-minded enough to print such an article is the kind of journal that I want to support."

His ancestors were pioneers and frontiersmen who felled many

a forest and subdued many a stony field. They helped to settle half a dozen towns in eastern Massachusetts and then moved on into the wilds of Connecticut, afterwards into Central New York, and still later into Southern and Central Michigan—always pushing the frontiers farther and farther back. How typical of the scientific frontiers conquered by their illustrious descendant!

Of his upwards of two hundred scientific contributions,<sup>1</sup> little need be said here; they are too well known throughout the world of biology to require detailed comment. The breadth of his scientific viewpoint is attested by the fact that he published in English, German and French in journals of such diverse interests as the *Centralblatt für Bakteriologie*, *Science*, *Reports of the Royal Horticultural Society*, the various series of the United States Department of Agriculture, *Compt. Rend. 1er Congrès International de Pathologie Comparée*, *Proceedings of the International Congress of Medicine*, *Journal of Cancer Research*, *Journal of Bacteriology*, *Proceedings of the National Academy of Sciences*, *Johns Hopkins Hospital Bulletin*, *Brooklyn Botanic Garden Memoirs*, *Archives of Dermatology and Syphilology*, *Phytopathology*, *Journal of Radiology*, *Journal of Infectious Diseases*, *Revue de Pathologie Végétal et d'Entomologie Agricole*, *Revue Botanique Appliquée et d'Agricultur Coloniale*, *Revue Générale des Sciences Pure et Appliquées*, *Journal of the Washington Academy of Sciences*, *Journal of Heredity*, *Scientific Monthly*, *American Naturalist*, etc. His textbook, an *Introduction to Bacterial Diseases of Plants*, and his three-volume monograph on *Bacteria in Relation to Plant Diseases* are well known to bacteriologists in general. It is not so well known that of the latter he had other volumes in preparation. The translation of Duclaux's life of Pasteur has won unstinted praise from scientist and layman alike.

In his scientific researches he had that rare ability of sensing when he should forge ahead and when he should call a halt. This trait is evidenced by his reply to a written query from a fellow scientist. "I was rather amused," he wrote, "at your

<sup>1</sup> A complete bibliography has appeared in the October, 1927, issue of *Phytopathology*.

questions concerning my investigations on peach yellows and my reasons for stopping. When I was a young man and came to the Department of Agriculture, I requested them to give me the most difficult problem which they had. They assigned me the subject of peach yellows. This was my reason for undertaking the investigation. The reason that I gave it up was that I was tired of butting my head against a stone wall." It is worthy of comment that the state of knowledge regarding this disease remains today essentially where Smith left it.

A history of the bacteriology of plant diseases could not be written with Smith left out of the picture; yet perhaps he will remain best known to biologists as a whole through his monumental investigations of the general cancer problem—in animals and man as well as in the realm of plant life. His position here is perhaps best expressed in the words of Dr. William H. Welch, addressed to Smith himself at the testimonial dinner given in his honor by the American Phytopathological Society in Philadelphia, December, 1926: "No one in our day has done more to bring these two great divisions of pathology into closer relation to their mutual advantage. . . . Above all, your studies of tumors of plants, which you have demonstrated to be of bacterial origin, have brought you into the field of oncology in its broadest aspects. Here you take your place in national and international congresses and associations devoted to cancer research or to medicine in general, and here your work is recognized as of the greatest interest and importance. While your name is associated especially with the championship of the parasitic theory of the origin of tumors, your studies of the mechanism of tumor formation, of problems of histogenesis, of formative stimuli and inhibitions of growth and other kindred subjects are scarcely of less importance. . . . We, too, on the medical side, as well as your own more immediate colleagues in plant pathology, have had opportunities of close association and have learned to admire you as a man inspired with the highest ideals of the searcher for truth in nature and devoted to this search with the heart, the methods and the loyalty of the ideal man of science." It need only be added that in 1924 Smith was elected president of the American Society for Cancer Research—a most unusual honor to

be accorded a man engaged primarily in the field of plant research.

Those interested in Dr. Smith's philosophy of life I can perhaps best refer to his address,<sup>2</sup> "Some Thoughts on Old Age," delivered as guest of honor at the annual dinner of the Botanical Society of Washington, in 1924, and epitomized in the following sonnet given at the close:

AT SEVENTY

Backward I look from the summit of the years  
At the rugged dusty way of toil and grime,  
From level distant plain of boyhood's prime,  
—Way strewn with hopes, with triumphs and with tears;  
And I am optimist, like him who hears  
Clear voices call from higher peaks of Time,  
Across the cloudy glens, and turns to climb  
What yet remains, with more of hopes than fears.  
I'm but a grain of sand upon Time's shore,  
Driven by wind and water evermore!  
And *millions* make but shifting dunes and bars!  
Yet I can read in every grassy sod,  
Divine great thoughts that sweep beyond the stars  
And make me one with Him who is our God!

To the world of science and to his friends it will never "be as if he had never been."

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UNIVERSITY OF PENNSYLVANIA

<sup>2</sup> Jour. Washington (D. C.) Acad. Sci., 14 (11): 231-238. 1924.

# BIOLOGIC STUDIES IN THE SPHAERIALES—I<sup>1</sup>

JULIAN H. MILLER

(WITH PLATES 21 AND 22 AND 3 TEXT FIGURES)

The fungi which constitute the object of this investigation are included in the Sphaeriales by Lindau (16). This order, together with the Perisporiales, Hypocreales, Dothideales, and Laboulbeniales, compose the Pyrenomycetes, an enormous assemblage of forms distinguished by the type of ascus conceptacle which is called the perithecium.

The orders of the Pyrenomycetes are separated by most mycologists in the following manner. The Perisporiales comprise forms in which the perithecia remain closed or have an atypical opening. The Hypocreales have fleshy, bright-colored or colorless perithecia which, though sometimes brown, are never black and hard. In the Dothideales the asci are formed in locules in a stroma, and true perithecia are lacking. In the Sphaeriales plainly differentiated leathery, hard, or carbonaceous perithecia occur with or without an accompanying stroma. Finally, the Laboulbeniales have perithecia, but lack a true mycelium.

The application of these separations has resulted in bringing together clearly unrelated fungi, due in part to a misconception of the fundamental differences between the perithecium and the stroma.

It should be emphasized then that in the Sphaeriales the asci are borne in perithecia, and this order is separated from the Dothideales by the fact that in the latter the asci are located in

<sup>1</sup> Also presented to the Faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

This investigation was accomplished under the direction of Dr. H. M. Fitzpatrick, to whom the writer wishes to express his appreciation for his suggestions and continued helpful supervision of the problem. The writer is also indebted to Dr. L. Massey for making available the herbarium and the facilities of the laboratory, and to Prof. H. H. Whetzel for much inspiration and encouragement in the prosecution of this investigation.

cavities or locules in a stroma. In reality this separation has been used only when there are two or more locules or perithecia in the stroma. Species in which only one locule is present have been placed in the Sphaeriales, there being no method known for differentiating an unilocular stroma from a true perithecium. This order, as generally delimited, contains consequently many forms which are properly placed in the Dothideales.

Many modern writers have recognized the presence of these unilocular forms in the Sphaeriales, and some, *e.g.* von Höhnelt (10, 11, 12, 13, 14, 15), and Theissen and Sydow (28, 29), have contributed a mass of evidence which will aid in the development of a more natural arrangement. While these investigators have uncovered characters that clearly distinguish unilocular stromata, they have failed to recognize the fundamentally important difference in development between the tissue constituting the boundary of the locule and a true perithecial wall, or the correlated difference in ascigerous development. This has led these investigators, along with Petrak (21) and Gäumann (7), into the error of assuming that within any given group of dothideaceous forms a perfect series of transitions into the Sphaeriales can be attained by selecting ones with consecutively thinner walls. According to them the unilocular form with an uniformly thin wall, even though the interior of the locule be of the dothideaceous type, falls in the Sphaeriales.

The purpose of this investigation has been to demonstrate differences in development between the Dothideales and the Sphaeriales, which hold for all forms, including those with one locule and those with one perithecium in the stroma; and to show that when the dothideaceous forms are removed from the Sphaeriales the remaining species will constitute a definite series of related forms.

### Explanation of Terms

**Stroma.** The vegetative matrix called the stroma, which functions in the storage of food for the development of the fructification which later arises in it, is a common and variable structure in the Ascomycetes.

The limits of the term stroma have been defined variously by different investigators. Orton (19) summarizes the concepts of



Persoon (20), Tulasne (30), Fuisting (5, 6), Ruhland (24), and others. Persoon introduced the term in connection with the genus *Sphaeria* and applied it to the structure in which the perithecia are borne. Tulasne employed it for the body in the family Xylariei, which forms first conidia, and after further development perithecia.

Fuisting and Ruhland distinguish different types of stromata in those groups of the Sphaeriales in which this structure contains more than one perithecium. The former writer, in the case of *Nummularia Bulliardii* Tul., distinguishes a hyaline, pseudoparenchymatous crust, found in the outer layers of the primary cortex of the host, which produces conidia. He terms this the **epistroma**, and says that it functions both in rupturing the bark and in producing conidia. Under this crust a **hypostroma** is produced, in which later perithecia are developed. Ruhland follows the conception of Fuisting, but uses instead the terms **ectostroma** and **entostroma**, respectively.

Ruhland recognizes further differentiation in the two layers. He designates the ostiolar disk by the term **placodium**; and since, in forms like *Diatrype disciformis* Fries, it originates from the entostroma, he characterizes this type as **entoplacodial**. He then traces transitions from the entoplacodial type to his **ectoplacodial** type—through which the entostroma becomes reduced and the placodium is formed in part at least from the ectostroma. Where both ectostroma and entostroma are present he terms the body **diplostromatic**, and where one only is present, **haplostromatic**. Therefore, with the types with reduced entostroma it is but a step to his haplostromatic type, in which the entostroma disappears entirely, and the perithecial initials develop within and near the base of the ectostroma. He says the Xylariaceae belong to this type, and thinks these are the highest forms in the Sphaeriales.

Wehmeyer (31: 579) defines the stroma as "an aggregation of vegetative mycelium not resulting from a sexual stimulus." He excludes the tissue composing the perithecial wall and centrum (ascigerous portion), the tissues of the apothecium, and the purely nutritive mycelium which is neither definitely aggregated nor coalesced. As sclerotia and other sterile masses, not associated with spore-bearing structures, are identical in their histo-

logical nature with the matrices of various compound fruit-bodies, they fall within the limits of the definition. Wehmeyer (31: 580) says, further, that he uses the terms ectostroma and entostroma to designate differences in structure and position and not in function, and defines them as follows: "An ectostroma in the Pyrenomycetes is that portion of the stroma which is formed on the surface of the bark, beneath or within the periderm, and which consists typically of fungous tissue only, except that when it is developed within the periderm it may contain the remnants of periderm cells, but never of the bark cortex cells. An entostroma is that portion of the stroma which develops within the cortical or woody tissue of the host or substratum, and is made up of components of both fungous and host tissue or substratum."

The writer feels that these definitions of ectostroma and entostroma are not comprehensive enough to include all Pyrenomycetes, or even all members of the Sphaeriales. Wehmeyer says that they designate differences in structure and position, but he uses them to designate differences in position only, *i.e.* ectostroma in periderm, entostroma in cortex. His definitions are clearly limited to forms in the Allantosphaeriaceae and the Diaporthaceae which occur on hosts with periderm, and are not applicable to forms which occur on monocotyledonous hosts, or on decorticated wood or leaves. Furthermore, in the Xylariaceae, while the entostroma is initiated in the cortex, or woody tissue of the host, it is composed of fungous tissue alone. This type of entostroma would not come within the scope of his definition.

According to Gwynne-Vaughan and Barnes (8: 1), a stroma is defined as follows: "In most cases the hyphae are richly branched; they elongate by apical growth, and, as a rule, spread loosely through the substratum; in some cases, and especially in relation to the fruit bodies of higher forms, they become woven into a dense mass which in section gives the appearance of a tissue, and is therefore described as pseudoparenchymatous; such a mass, when not forming part of a single fructification, is termed a stroma; . . . ." This definition is applicable to forms in which there is more than one locule or perithecium in the stroma, and evidently does not include the forms with only one locule or perithecium in the stroma.

In this paper the term stroma will be used to include fungous bodies which are formed of coalesced hyphae, which do not arise as a result of a sexual stimulus. There is apparently a tendency toward the reduction of the sexual apparatus in the Ascomycetes. Nevertheless, the ascospore has always been considered the sexual spore. Its sexual nature has been demonstrated in many cases. Therefore, to the writer, it seems logical to term the body which arises from the archicarp and bears ascospores the sexual fructification, in contrast to the stroma. In the Sphaeriales the body (perithecium) which arises from the archicarp (including wall, ascogenous hyphae, asci, paraphyses and periphyses) is a distinct generation in the life cycle of the fungus, and is just as truly distinct from the other generation (mycelium and stroma) as the sporophyte is distinct from the gametophyte of a higher plant. In *Sclerotinia*, the sexual fructification (the apothecium) arises from an archicarp in the sclerotium. The sclerotium is therefore a stroma. In *Claviceps* the perithecia are the sexual fruit-bodies and the rest of the structure, including the sclerotium, is stromatic. In *Rosellinia aquila* (Fries) De-Not. (TEXT FIGURE 1) an extensive stroma often develops under the

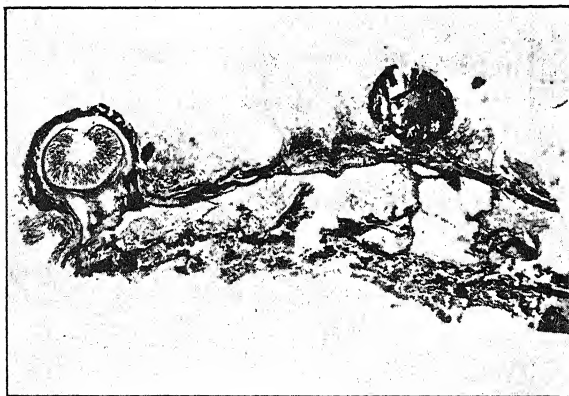


FIG. 1. *Rosellinia aquila* (Fries) De-Not. Longitudinal section through a mature perithecium and stroma. This photomicrograph shows a perithecium in the upper part of an entostroma and oriented under a definite ectostroma.

periderm. This ruptures the bark in places, and develops further perpendicularly to the substratum. An archicarp then arises in

the external part and develops into a perithecium. It could not be said that the portion of the stroma that grows out through the ruptured bark, and in which the perithecium is formed, is pushed out as a result of a sexual stimulus. The sexual stimulus is in the archicarp, and the latter arises after the stroma has grown out. In *Claviceps*, *Cordyceps*, and certain species of *Xylaria*, which arise from a sclerotium, the archicarps are found in the periphery of the vertical structure and not in the sclerotium. Therefore, it seems illogical to say that the vertical structure arises as a result of a sexual stimulus. This structure is merely a continuation of the sclerotium and the whole tissue is a stroma.

The terms ectostroma and entostroma are useful in dealing with the Sphaeriales. The former will be limited to the part of the stroma first formed in or on the periderm, or on the wood when the bark has been removed, which functions in rupturing the bark when the latter is present and which usually functions in producing conidia. The term entostroma will be applied to the portion of the stroma which develops under this and bears perithecia in its periphery. The entostroma in most of the forms studied by Wehmeyer consists of stromal elements mixed with wood and, in the sense of a pseudoparenchymatous tissue, is certainly not a true stroma. It often has been termed a valsoid stroma. The writer considers it a primitive type of entostroma. In the Xylariaceae the effused forms, such as *Nummularia*, represent the minimum of entostromatic development, and certain species of *Xylaria* the maximum. In fact these two genera differ only in the amount of entostroma. The ectostroma is the primary outer layer in both. Also in the Allantosphaeriaceae and Diaporthaceae, as compared with such a form as *Hypoxylon coccineum* Bull., there is very little entostromatic development.

In the Sphaeriales and Hypocreales, forms such as *Rosellinia* and *Melanospora*, in which the perithecia are single, have not been previously considered as stromatic. In the early development the archicarp gives rise to a coiled ball of fine hyphae which forms the perithecial wall, asci, paraphyses, and periphyses. The surrounding tissue, which is large-celled and pseudoparenchymatous in type, and which may exist later merely in a fragmentary

state on the outside of the wall, is certainly stroma. In fact the perithecium in these two orders is, contrary to previous conceptions, apparently always formed within a more or less well-developed stroma.

In the Dothideales the sexual fructification consists of the group of asci and the hymenial layer from which they arise. The rest of the tissue making up the structure is clearly stromatic. This has never been a matter of dispute.

**The perithecial wall.** The Sphaeriales have been considered by all mycologists as having an ascigerous cavity bounded by a definite perithecial wall, in contradistinction to the Dothideales, where the cavity lacks the wall. In compound fructifications this difference has been clearly recognized, but the unilocular forms in the Dothideales, and the uniperithecial ones in the Sphaeriales, have resulted in much confusion.

Von Höhnelt (10, 11), Theissen and Sydow (29), and others have attempted to consider, together with unilocular forms, those plurilocular forms in which each locule fills an arched-up portion of the stroma resembling externally a perithecium. They segregated these in the Pseudosphaeriaceae v. Höhnelt (Pseudosphaeriales Th. & Syd.). They adopt as a basis for the separation the character<sup>2</sup> of the interior of the so-called cavity. Neither these writers nor Gäumann (7) take into consideration the ontogeny of a true wall. Gäumann for instance places *Botryosphaeria* in the family Dothioraceae of the Myriangiales, and asserts that the asci are arranged in locules in the stroma. Then in regard to the development in this family he says (page 214) that such forms as *Botryosphaeria Bakeriana* Rehm, *B. Quercuum* (Schw.) Sacc., and *B. Ribis* G. & Dug. have attained the highest level, because here the locules appear to be in process of separation, and finally in some cases contain only one locule resting on a stromal base. He then says these latter, isolated forms have their own walls, and so have become perithecia. Clearly, then, his definition of a wall, like that of most mycologists, is based on a gross morphological conception, which does not take into consideration the origin of the wall. The writer has found that in *B.*

<sup>2</sup> Von Höhnelt terms the mass of asci, etc., which fill the cavity of the perithecium, the perithecial nucleus, but the writer will use the term perithecial centrum or locule centrum, avoiding this incorrect use of the term nucleus.

*Ribis* the size and form of the stroma are dependent on characters of the substratum, such as thickness of bark, firmness, etc. Where the bark is thick the stromata are thick with many locules, and where the bark is very thin the stromata become increasingly thinner with a tendency to be unilocular. Gäumann (7: 284) says, further, that among the higher *Scolecosporae* of the *Hypocreales* stromatic forms without perithecial walls arise from stromatic forms with solitary perithecia. This causes him to question the correctness of recognizing the group *Dothideales*. It would seem from this that he thinks that the presence or absence of a true wall is of no systematic significance. In the *Hypocreales* the writer has examined no species in which the ascigerous cavity lacks a true wall. The species placed in the genus *Ophiodothis* of the *Dothideales* by Saccardo (25: 652), including species of *Dothichloe* Atk. and *Myriogenospora* Atk., have distinct perithecial walls, and are related to *Balansia*, *Hypocrella*, *Claviceps*, and *Cordyceps* in the *Hypocreales*. Theissen (28: 187) has called attention to this fact.

To the writer the perithecial wall seems to afford a character which is definitely correlated with characters of the perithecial centrum; and the forms possessing it stand in sharp contrast to the dothideaceous fungi in which it is lacking. The characters that accompany the lack of a perithecial wall are the presence of pseudoparenchyma in the centrum, absence of paraphyses and periphyses, the convex to flat or concave form of the hymenial layer, and the lysigenous type of the opening. When there is a true wall there is no pseudoparenchyma in the centrum, there are true paraphyses and periphyses, concave hymenial layer, and the ostiolum is shizogenous in type. The wall in the *Sphaeriales* is histologically and ontogenetically different from the tissue of the stroma. The writer will define it as the specialized tissue, which arises from the archicarp, and from the beginning encloses the ascigerous centrum. None of the cells of the wall are derived from those of the stroma other than those of the archicarp. Certain previously published evidence corroborates this view.

Gwynne-Vaughan and Barnes (8: 234, FIG. 186) represent a single hypha, the archicarp, which initiates the whole perithecium in *Xylaria polymorpha* (Pers.) Grev. They say: "If the stroma

of *Xylaria* or *Hypoxylon* is sectioned during the conidial stage, nests of small hyphae are found, and form the first indication of perithecia (FIG. 185). Still earlier a stout hypha with large nuclei, presumably an archicarp, is recognized." The writer will show later that in *Hypoxylon* the primary coil, or archicarp, arises as a single hypha in the stroma. In its later development its external cells coalesce to form the perithecial wall, while its central cells give rise to asci and paraphyses. Therefore, in a true perithecium, the layer of asci, including the paraphyses, is directly connected with the wall. The writer has often succeeded with macerated stromata in getting the perithecium out intact and free from stroma. If the wall were only a modified inner layer of the stroma, that would be impossible.

In the Erysiphaceae also the wall arises from the archicarp. Hein (9: 391) says: "The enveloping hyphae arise just below the septum which cuts off the oogone from the basal cell and at a corresponding level from the basal cell of the antherid."

Also the apothecium of the Discomycetes arises entirely from the archicarp. In regard to *Pyronema confluens* Tul., De Bary (1: 208) says: "Copiously branched hyphae begin to shoot out from the sterile branches of the archicarp, and from the whole of the rest of the basal region of the rosette to form the envelope portion of the sporocarp."

This explanation of the significance of the wall has been given in detail, because only by having its origin clearly in mind can one easily separate unilocular dothideaceous forms from those with true perithecia.

The genus *Guignardia* affords a case in point. This genus has been placed in the family Mycosphaerellaceae of the Sphaeriales, and the perfect fruit-body has been thought to be a true perithecium. Theissen and Sydow (29) noted that the asci arise in a homogeneous pseudoparenchyma in the Mycosphaerellaceae in the same manner as in the Dothideales, and they placed this family in their order Pseudosphaeriales. Von Höhnelt (12: 629) says: "Die Gehäuse der Sphaerella-arten sind keine Perithezien sondern kleine (meist) einhausige, peritheziennährliche Stromata. Solche Gebilde nenne ich Dothithezien." In regard to the origin of the asci in *Guignardia*, Reddick (23: 311) says: "Such peri-

thecia are surrounded by the usual thick, black, pseudoparenchymatous covering. This pseudoparenchyma becomes thinner walled inwardly, so that the whole interior of the perithecium is filled with it. In the stained sections there are scattered here and there, near or a little below the center of the perithecium, little dots, of much deeper staining quality, which in well bleached preparations are seen to be individual cells when examined with an immersion lens. . . . When activity begins, the ascogenous cell elongates by pushing its way upward, though at the very first it seems to take the path of least resistance and may grow in a longitudinal direction for some distance." He found very young stromata composed of homogeneous pseudoparenchyma, which he termed pycnosclerotia, and he thought they were sporeless pycnidia, which may eventually develop into perithecia. In examining his preparations the writer finds, as stated above, that in the earliest stage seen the so-called perithecium consists of a homogeneous pseudoparenchymatous matrix, in which a little below the center the archicarp arises. At this stage the condition is identical with that shown by the writer for *Hypoxylon* (PLATE 22, FIG. 7). But in *Guignardia* (PLATE 21, FIG. 4) as the asci develop, the tissue directly above disintegrates, and the asci push up between the fragments. In the Sphaeriales (PLATE 22, FIG. 6) the archicarp very early gives rise to a wall, which definitely shuts out all the pseudoparenchyma of the stroma. In *Guignardia* the archicarp gives rise to no wall, and so has exactly the same type of development found within the Dothideales.

**The ostiolum.** There seems to be no general agreement among mycologists as to the limits of this term. Some would apply it to any pore of an ascigerous or pycnidial fructification through which spores are liberated. Others apply it to the papilla or neck of the conceptacle.

Wehmeyer (31: 582) says: "The ostioles are merely the erumpent portions of the perithecial necks." Von Höhnelt (15: 138), in regard to the Coronophoreen, characterizes them as having no ostiola, and places them in the Allantosphaeriaceae, all the other members of which have ostiola. Toro (26: 40) created the order Pseudoperisporiales on two genera, *Porostigme* Syd. and *Pseudoperisporium* Toro. He says: "The order



differs from the Perisporiales in having perithecia with definite ostiola." If the conception of an ostiolum is of such taxonomic value, it should certainly not mean different things to different mycologists.

De Bary (1: 190), speaking of perithecia, says: "They are bounded on the outside by the wall, which encloses an ascigerous hymenium, and are furnished in the full-grown state with a narrow aperture or ostiole, which is a canal passing through the wall, and serving for the discharge of spores. . . . The ostiole is not formed till the development is more advanced, and it appears as an intercellular passage in the originally closed tissue; it is partly schizogenetic by the separation of persistent tissue elements in consequence of unequal growth, partly lysigenetic by the dissolution of a strip of tissue lying originally in the canal."

Most writers seem to agree that the opening in the Dothideales does not constitute an ostiolum. Gäumann (7: 284), in discussion of this order, says that, from the lack of a special perithecial wall, there follows the lack of an ostiolum, and the summit of the locule is always formed through a definite part of the stroma regardless of the shape of the summit. So he thinks that the lysigenously formed pore in the summit of the stroma does not constitute an ostiolum. Theissen and Sydow (29), in regard to the Pseudosphaeriales, say that an ostiolum is not present. They apply this point of view then to such forms as those in the Cucurbitariaceae, Pleosporaceae, Mycosphaerellaceae, as well as to those in the Dothideales. Blain (2: 17), in his study of the dothideaceous stromata, says: "No definite ostiole has been found in the study of the fungi involved in this paper except those which obviously belong to the Sphaeriales."

The writer will consider as an ostiolum the canal passing through the papilla, or neck of the perithecium, and terminating in a pore. It is lined with minute periphyses, which are outgrowths of basal cells in the neck wall and have free ends. The pore is formed by the pulling apart of wall tissue at this point due to unequal growth, and the canal is formed by the upward growth of the wall. It is never formed lysigenously as in the Dothideales. In this conception the term ostiolum is clearly limited to the perithecium of members of the Hypocreales and Sphaeriales.

The latter part of De Bary's definition is applicable to the Dothideales and Pseudosphaeriales. In both cases there is a dissolution of a strip of pseudoparenchymatous tissue directly above the asci, which results in an opening, but the tissue lining the canal is histologically stroma, and not wall tissue as in the Sphaeriales. These two methods of forming the canal are so distinct that it would certainly not be accurate to use the term ostiolum for both.

**Paraphyses.** This term has been applied to sterile threads lying between the asci in a parallel position. However, since such threads are known to arise from different sources, it is necessary to define the term more definitely.

Petrak (21: 67), in discussing the evolution of paraphyses, considers those found in both types of centra, *i.e.* the Pseudosphaeriales or the Dothideales on the one hand, and the so-called Diaportheen<sup>3</sup> centrum on the other. The first type of centrum he divides into three categories: (1) Paraphyses completely lacking, examples *Mycosphaerella*, *Sphaerulina*, and *Guignardia*. (2) Paraphyses more or less thread-like and cellular, entirely atypical, formed from compressed parts of the ground tissue. As examples he cites *Wettsteinia*, *Pseudosphaeria*, *Botryosphaeria*, *Dothiora*, *Pleospora* sp., and *Leptosphaeria* sp. He calls these paraphysoiden, and says they are entirely primitive forms of paraphyses. (3) Paraphyses more or less strongly developed and branched, not at all, or scarcely gelatinizing, grown above to the covering tissue of the perithecial membrane. As examples, he designates *Leptosphaeria* sp., *Melanomma* sp., *Trematosphaeria* sp., *Massarina*, *Massaria*, *Pleomassaria*, and many others that he considers as belonging to the Sphaeriales, as well as numerous dothideaceous fungi.

He follows this with his Diaportheen type. In this type of centrum there is no pseudoparenchyma. He divides this into three groups: (1) Pseudoparaphyses completely lacking. Here he puts most of the genera of von Höhnel's (13: 631) Diaportheaceae, also *Valsa*, and *Melanconis* sp., "but no single Dothideales form." (2) Pseudoparaphyses rather numerous, cellular, rela-

<sup>3</sup> The term "Diaportheen centrum" is used here in the broad sense to include perithecia with true paraphyses and asci lining the base and sides, and genuine ostiola lined with periphyses.

tively broad, usually delicate, and at early stages strongly gelatinizing. He gives, as examples of this type, many species of *Melanconis* and *Pseudovalsa*. (3) Pseudoparaphyses more or less, often very numerous, not distinctly cellular, threadlike, not easily gelatinizing, free above. As examples displaying this last type of pseudoparaphyses he cites *Hercospora*, *Rosellinia*, *Hypoxylon*, and *Xylaria*. He calls these pseudoparaphyses of his third type metaphyses.

In his first division he has a series of transitions from no paraphyses to paraphysoiden, to so-called genuine paraphyses. However, as he says, all of these are connected to the perithecial membrane above the asci. In his second type, the Diaportheen, all arise from the ascal layer, and are free above.

*Guignardia Bidwellii* (Ellis) Viala & Ravaz (PLATE 21, FIG. 4) represents Petrak's first type; also *Dothidea collecta* (Schw.) Ellis (TEXT FIGURE 2). The tissue above the asci is stroma in process of dissolution, and there are no paraphyses. The condition in *Chaetosphaeria phaeostroma* (Mont.) Sacc. (PLATE 21, FIGS. 2, 3) and also *Dibotryon morbosum* (Schw.) Th. & Syd. (PLATE 21, FIG. 6) corresponds to his second and third types. The strips of tissue are connected at the top and are plainly to be seen as only strips of dissolving pseudoparenchyma. *Hypoxylon Howeianum* Peck. (PLATE 22, FIG. 5) is of the Diaportheen type, and has what Petrak calls metaphyses.

It is evident that Petrak has not considered the origin of his so-called dothideaceous type of paraphyses. He says (21: 67) that pseudoparaphyses have free ends, while genuine paraphyses are grown above to the perithecial cover. The illustrations cited above show plainly that these strands are only stromal remnants. Moreover, no part of the stroma, which is external to the ascogenous layer, could by any series of transitions become genuine paraphyses. In the Discomycetes true paraphyses are never found growing down from the top of the apothecium. There is no way that one can reconcile these stromal parts with paraphyses. Petrak is merely attempting here to show by means of the pseudoparenchymatous threads hanging from the top of the centrum that the Pseudosphaeriales grade into the Sphaeriales. The separation between paraphyses in the Sphaeriales and

stromal remnants in the Dothideales is just as exact as between the wall in one case and the stroma in the other.

True paraphyses, then, are sterile hyphae, which arise in the ascogenous layer, and have free ends converging towards the ostiolum. They appear before the asci, and usually gelatinize at maturity.

### Comparison of Development of the Dothideales and the Sphaeriales

The illustrations of *Chaetosphaeria phaeostroma* (Mont.) Sacc. (PLATE 21, FIGS. 1, 2, 3) show a development typical of the Pseudosphaeriales of Theissen and Sydow (29). First a pseudo-parenchymatous stroma develops; then an archicarp appears slightly below its center. As this grows the ascogenous hyphae and asci appear, and the latter grow upward in the pseudo-parenchyma. This tissue appears to undergo a chemical dissolution as the asci increase in size. Its cell walls become thinner, and the black coloration in them dissolves. Apparently, this part of the stroma acts as nurse tissue in the same manner as do tapetal layers surrounding pollen grains in the Spermatophyta. The formation of the opening is accomplished by the same type of chemical dissolution. The dark-walled external cells directly above the developing archicarp become hyaline and thin-walled and gradually break apart. The actual break is probably caused by the expanding hymenial layer. With the low power of the microscope the canal appears as a typical ostiolum, but when examined with the high power it is seen to contain, instead of paraphyses, merely cell fragments.

The method of development typical of the Dothideales is to be seen in the longitudinal section through the stroma of *Dothidea collecta* (TEXT FIGURE 2). The asci develop from a convex placenta, in the same manner as in *Mycosphaerella Fragariae* (Schw.) Lind., growing upward in the stroma, and at maturity the stroma disintegrates to form an irregular opening through which the spores escape. In *Guignardia Bidwellii* (PLATE 21, FIG. 4), the same type of development as that in *Dothidea collecta* is shown, except in that the ascogenous layer is flat instead of convex. In all cases the stroma develops first, and the archicarp

later. There is no indication of a perithecial wall, the centrum being solidly pseudoparenchymatous.

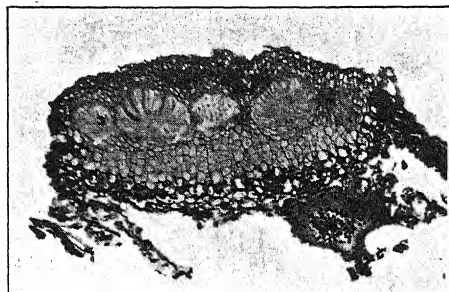


FIG. 2. *Dothidea collecta* (Schw.) Ellis & Ev. Longitudinal section through a mature stroma showing groups of asci in locules.

Blain (2: 17) says in regard to the Dothideales: "Nearly all of the fungi examined possessed a concentric layer of thin, hyaline compressed cells around the periphery of the locule, the lining." This is not comparable to a wall in any sense. It is due to the dissolution process, and to pressure from the growing asci, to which these cells are subjected.

This dothideaceous type of development is also seen in *Dibotryon morbosum* (PLATE 21, FIG. 6), and in *Leptosphaeria Doliolum* (Pers.) Wint. (PLATE 21, FIG. 5), the threads simulating paraphyses being stromal remnants as previously explained.

Nichols (20: 316) says in regard to the development of *Teichospora obducens* (Fries) Fuckel and *Teichospora sporadica* Atk.: "A single cell of the mycelium by successive divisions and growth forms a solid sphere of parenchymatous tissue. Certain of the interior cells of this tissue become enlarged and differentiated into asci." This is typical of dothideaceous development. The cell that initiates the stroma, in locular forms, cannot possibly be considered the archicarp. That organ arises later.

The type of development found in the Sphaeriales produces Petrak's "Diaportheen centrum." The archicarp always develops in a stroma, whether there be a single perithecium or many. The wall forms early, shutting out all of the stromal parenchyma.

In TEXT FIGURE 1, *Rosellinia aquila* (Fries) De-Not. is pictured to provide an example of a single perithecium enclosed in a stroma.

This form has not been considered as one of the stromatic Sphaeriales. The stromal part, which encloses the perithecium, has been thought of as a part of the perithecial wall. This, however, is ectostroma, under which the perithecium develops in the same manner as in *Hypoxylon*. The stroma is hard and carbonaceous, but the perithecial wall is not. The illustrations of perithecia of *Neurospora* by Shear and Dodge (27: Pl. 4, A, B, C) show typical perithecia, in which there is a definite wall, and also a thin pseudoparenchymatous layer on the outside, just as in species of *Rosellinia*. This pseudoparenchyma is stroma. Histologically it is the same as the stroma in such compound fructifications as in *Hypoxylon*. All the tissue that does not arise from the original coil is stroma. Apparently all the so-called nonstromatic forms in the Sphaeriales are in reality stromatic.

The development of *Hypoxylon Howeianum* Peck. is illustrated on PLATE 22. A coiled archicarp arises in the periphery of the entostroma (FIGS. 4 AND 7). By further growth this gives rise to a ball of hyphae. The outer hyphae of this knot are smaller in diameter than those on the inside. Next, the outer threads coalesce to form a globose wall. Inside of this wall are loosely coiled segments of large diameter, which later give rise to ascogenous hyphae. These have been called "Woronin hyphae." The wall definitely shuts out the stroma. The fertile segments gradually settle out, forming a peripheral layer lining the wall. At this stage the apical region of the wall begins to grow upward, cone-like. This gradually grows out through the ectostroma (FIG. 3) and forms the ostiolum in the manner described above under the explanation of ostiolum. The mature perithecial centrum is then surrounded by a wall (FIG. 5), the sides and base of which are lined with asci and paraphyses, and the ostiolar canal is lined with periphyses. This fully equals Petrak's *Diaportheen* type of centrum as described previously.

This method of development corresponds closely with that of *Xylaria polymorpha* (Pers.) Grev., as described by De Bary (1: 216): "The primordia of the perithecia make their appearance in the form of small spherical bodies, which lie in the medulla close beneath the black rind, and are at once distinguished from the medullary tissue by containing no air and therefore being

transparent. They are formed of a closely woven mass of slender hyphae, which are much thinner than the hyphae of the original tissue and must therefore be a new formation in it. In somewhat older specimens an irregular large-celled coil of tissue is found lying in the middle of the sphere. The spheres now increase in size in the direction of the medulla, the shape, structure and position remaining the same. Then a dense tuft of straight hyphae, in the shape of a broad truncated cone, shoots forth from the part which abuts on the rind and elongates in the direction of the rind, which is first bulged out a little and then gradually pierced through, so that the extremities of the hyphae project above the surface. The young perithecium has meantime become egg-shaped, its broader portion lying in the medulla being the future basal part, while the narrow end which is wedged into the rind is the future neck with the ostiole."

Modern investigators have added to our knowledge of the early stages of development within the centrum.

Brown (3: 4) says of *Xylaria*, "Soon a definite perithecial wall is to be seen (FIG. 10). As this grows it seems to spread so as to make more space within; the Woronin hyphae appear to lie loosely within the space enclosed. . . . As the segments enlarge the ends tend to become rounded so that the connection between segments is very slight, and they finally separate completely. Each seems to be an independent structure. The hyphae during the stages figured in 10 and 11 seem to be loosely coiled in the large space in the center of the developing perithecium but a little later they come to lie near the perithecial wall. . . . Later some of them send out branches and these branches give rise to ascogenous hyphae (p. 5). Following the stage shown by FIGURES 23 AND 24, with the enlargement of the perithecium, there is an increased growth of threads from the inner portion of the perithecial wall. . . . They extend from the wall and gradually fill the space within. These ingrowing hyphae form the periphyses and paraphyses (p. 7)."

Lupo (17: 494), in regard to the development of *Hypoxylon coccineum* Bull., says: "The formation of the perithecium is initiated by the massing of the hyphae into a circular knot, within the center of which the Woronin hyphae differentiate."

Dawson (4: 255), in regard to the development of *Poronia punctata* Fries, says: "The mature perithecium consists of a very definite wall of closely interwoven hyphae lined with a smaller-celled hymenial layer, whence arise the very numerous club-shaped asci, intermingled with numerous paraphyses. The somewhat long neck which opens by an ostiole to the exterior, is lined by delicate periphyses, which more or less completely fill the cavity leading into the perithecium."

The fundamental point of difference then between the Sphaeriales and the Dothideales is not the presence of pseudoparenchyma in the centrum in the latter, but the presence of the wall in the former, which determines that stromal tissue may not be enclosed in the centrum. If one could eliminate the stroma, it could be said in truth that in the Dothideales the ascal layer is gymnocarp from the beginning, and in the Sphaeriales angiocarp.

#### Discussion of the Pseudosphaeriales Question

Von Höhnelt, Theissen, and Sydow were the first mycologists to recognize that there are unilocular forms in the Sphaeriales with a different type of development from the remainder. Von Höhnelt (10, 11) founded the family Pseudosphaeriaceae on the genera *Wettsteinia* and *Pseudosphaeria*, and separated it as follows: stromata small, sunken, perithecium-like, with several locules standing near one another, each of which contains a single ascus. Theissen and Sydow (29) raised this family to ordinal rank. They say this order is recognized for sphaeriaceous fungi whose asci are separated by thin pseudoparenchymatous strands; and each ascus cavity is accordingly demonstrated to be a "monasker Lokulus," and the entire visible conceptacle as a stroma with many locules. They found many forms in the Sphaeriales that had this pseudosphaeriaceous centrum. The condition was considered to be related to that in *Myriangium* and *Plectodiscella*, where each ascus rests in its own locule. Also the Dothideales, *sensu strictu*, were seen to have this type of centrum. These writers, therefore, created the group Dothidiineae to comprise the three orders, 1. Myriangiales, 2. Dothideales, and 3. Pseudosphaeriales. They (29: 5) say that these orders are united by the common basic character of one-ascal locules. The last two orders



were separated as being stromatic (Compositae) and simple (simplices), respectively. In the Pseudosphaeriales they placed the following families: Epipolaeaceae, Parodiellaceae, Pleosporaceae, Cucurbitariaceae, Botryosphaeriaceae, Pseudosphaeriaceae, and the Sphaerellaceae.

Von Höhnelt (11: 634) considered the Pseudosphaeriaceae as being a connecting link between the Sphaeriales and the Dothideales, but says they remind one of the Myriangiaceae, due to the fact that the locules contain single asci.

Petrak (21) made a comparative study of a great many forms that von Höhnelt or Theissen and Sydow had placed in the Pseudosphaeriaceae von Höhnelt (or Pseudosphaeriales Th. & Syd.), and says that neither von Höhnelt nor Theissen and Sydow grasped their true meaning. After studying the species in the genera of the family Pleosporaceae, he says (21: 48) that with the species of the genera *Pleospora*, *Pyrenophora*, and *Leptosphaeria* the development of the perithecium of the typical Sphaeriales from a dothideaceous stroma can be followed very beautifully. The gradual formation of the ostiolum goes hand in hand with the changing of the stromatically formed wall into a perithecial membrane typical of the Sphaeriales, and with the increase in the number of asci there follows the development of typical paraphyses from the pseudoparenchymatous centrum tissue of the dothideaceous stroma. Further (21: 64), he says that von Höhnelt's Pseudosphaeriaceae are nothing but the primitive forms from which the Sphaeriales have developed, and that they bind the Sphaeriales directly with the Dothideales.

He brings this transition about through four developmental stages as follows:

1. Ostiolum still to be considered as a small, papillate extrusion of the conceptacle. Conceptacle wall very thickly pseudoparenchymatous, of rather homogeneous structure, differentiated into a dark colored outside crust, and a hyaline pseudoparenchymatous ground tissue. Asci not numerous, but very thick. Ground tissue still at maturity very plainly pseudoparenchymatous. . . . *Wettsteinia*, *Pseudosphaeria*.

2. Ostiolum distinct, however atypically developed; that is, remaining closed, but at maturity breaking out more or less.

Perithecial membrane strongly differentiated. Asci somewhat numerous, elongate, or thick clavate. Centrum tissue at maturity more or less threadlike, not plainly cellular.... *Pyrenophora phaeocomes*, *P. trichostoma* and *Pleospora herbarum*.

3. Ostiolum entirely typical; that is, at first completely closed, opening late, through partial gelatinous absorption of its tissue. Conceptacle wall still rather thick, more or less plainly differentiated in two layers. Asci numerous, slender. Ground tissue in the mature condition scarcely to be distinguished from typical paraphyses.... *Leptosphaeria Doliolum*.

4. Ostiolum typical, opening through a more or less round pore. Perithecial membrane composed of from a few to numerous layers of bright colored to dark colored, more or less compressed cells, membranaceous, leathery, or carbonaceous, never sclerotial. Asci very numerous, slender, centrum tissue composed of typical, more or less branched, robust, usually numerous paraphyses.

It is impossible for the gap between the Dothideales and the Sphaeriales to be bridged over in this manner. The fact that forms can be found with consecutively thinner walls does not mean that a membrane which is histologically stroma can ever be a true wall, nor can an ostiolum in the sense of Petrak by any series of transformations ever become a genuine ostiolum. The latter could exist only in the apex of a wall formed from the archicarp in the manner previously described in this paper for *Hypoxyton Howeianum*. Intertheccial tissue (stromal remnants) is found in all of Petrak's pseudosphaeriaceous fungi, and that very fact shows that there is no continuous wall as in the Sphaeriales. Any species which lacks a true wall necessarily has stromal tissue above the ascial layer, and so definitely belongs in the Dothideales.

Von Höhnelt considered that a fruit-body with a thin wall and an apical papillum constituted a genuine perithecium. He separated the genus *Leptosphaeria*, all of whose species have the dothideaceous type of centrum, into three genera. He (14: 135) first divided the genus into a dothideaceous *Leptosphaeria* Ces. & De-Not., based on the type, and a sphaeriaceous *Leptosphaeria*, which he put in *Nodulosphaeria* Rab. Later (15: 158), he distinguished a pseudosphaeriaceous *Leptosphaeria*, which he named

*Scleropleela*. He also failed to take into consideration the primary separation between the Dothideales and the Sphaeriales; *i.e.* the wall character.

It is now evident that von Höhnelt, Theissen and Sydow, and Petrak, while recognizing in part the characters of the unilocular forms, fail to agree on the limits of the Pseudosphaeriales, on the species and genera they include in the group, and in their conception of the morphology and homology of certain characters, *e.g.* wall, ostiolum, and paraphyses, which are of fundamental importance. Neither the taxonomic position of these forms nor their morphologic characters have been satisfactorily treated by any of these investigators. The unsatisfactory character of their work on the Pseudosphaeriales has been due to their failing to distinguish between pendant strips of partially dissolved stromatic pseudoparenchyma, and to their confusing the perithecial wall with the stroma.

It is impossible to make these forms transitions between the Dothideales and the Sphaeriales. None of them have a true wall, nor true paraphyses, and all have stromal parenchyma in the centrum. All of the characters found in the Pseudosphaeriales are common to members of the Dothideales. Therefore, they should be placed in the Dothideales, and it would seem that their position there should be determined by characters found in the centrum, such as characters of the ascus and ascospore and characters of the ascal hymenium. Such characters as the thickness or thinness of the stromatic wall or of the number of locules in the stroma apparently have no value.

#### The Relationship of the Myriangiales to the Dothideales and Pseudosphaeriales

According to Theissen and Sydow (29) the Myriangiales, Dothideales and Pseudosphaeriales are united by the common, basic character of the uniascal locule. Petrak (21: 61) points out that this is true in a strict sense for *Myriangium*, but not for members of the other two orders. However, he derives the latter from simple Myriangiales by increasing the number of asci from one to many, increasing the ascal plectenchyma in a horizontal direction in the stroma, and decreasing the thickness

of the outer stromal layer. Next he has the ascogenous plectenchyma increase in a manner to bring forth numerous asci, the outside crust becomes the perithecial membrane, and the papillate primitive condition changes into an ostiolum, the centrum pseudoparenchyma develops into paraphyses, and he has developed a genuine perithecium.

Gäumann (7: 209) would also derive the Pseudosphaeriales from the Myriangiales, and in fact makes the order Myriangiales comprise the families Myriangiaceae, Saccardiaceae, Dothioraceae, and Pseudosphaeriaceae. He thinks the genus *Kusanoopsis* represents the most primitive form. It forms dark cushions on leaves. The stroma is composed of homogeneous pseudoparenchyma, with no special rind. The asci are scattered in several layers over the entire interior of the stroma, with the exception of a sterile foot which projects into the mesophyll. The asci are spherical, and in individual locules. His second family differs from the Myriangiaceae only that its asci are no longer regularly formed in several layers, but are arranged in a single layer, which usually lies directly under the surface of the stroma and parallel with it. He says that they are not well known, but if one places the asci of *Kusanoopsis* in a single layer one can acquire an idea of their form. One might as fairly say, if he placed the asci of *Myriangium* in one group in the stroma he would have *Guignardia*, *Dothidea* or any other dothideaceous form. The jump between *Kusanoopsis* and the Dothideales, where the asci arise in a single layer, is far too great to warrant the placing of the Dothideales in the Myriangiales.

In TEXT FIGURE 3, a longitudinal section through the stroma of *Myriangium Duriae* Mont. is shown. The asci are distinct with definite pseudoparenchyma separating them. The writer sectioned material of various ages and could find no indication that the asci arise from a common archicarp. It certainly appears as though each ascus arises from its own archicarp, and that these initials arise at irregular intervals in the stroma. In this case then the asci are truly unilocular. In PLATE 21, FIG. 5, *Leptosphaeria Doliolum* (Pers.) Wint. is illustrated. Here the asci arise from a common hymenium and grow up into the pseudoparenchyma of the stroma. As they elongate, the cells

separate into thin strands which hang down between the asci at maturity. The writer has sectioned material of *Pleospora herbarum* (Pers.) Rabh. and finds the situation not at all comparable to Gäumann's figure (7: FIG. 145) of this fungus. In his

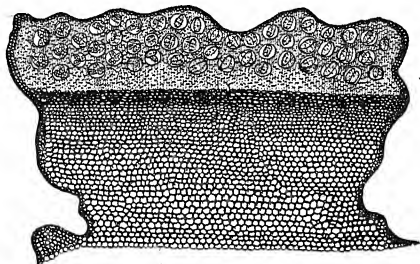


FIG. 3. *Myriangium Duriaei* Mont. Longitudinal section through an ascigerous portion of the stroma, showing single asci in stromal locules.

drawing the pseudoparenchymatous strands of interthelial tissue are shown connected at the top and bottom of the locule, apparently putting each ascus in a single pocket. The writer finds the ascial hymenium to be continuous at the base as in *Leptosphaeria Dolium*. It is impossible to homologize each ascus and its interthelial tissue here with the unilocular condition in *Myriangium*. The whole centrum in *Leptosphaeria* and *Pleospora* has a common origin, and is therefore homologous with the single ascus pocket of *Myriangium*. If the single archicarp in *Myriangium* could be conceived as continuing its growth and developing a horizontal layer in the stroma from which numerous asci then arose, and should thus evolve a dothideaceous type, then with equal logic it would be possible to think of the locule of *Myriangium* as having been derived by reduction from a typical dothideaceous locule containing many asci. An outer covering which is clearly stromatic cannot give rise by evolution to such a wall and ostiolum as that found in the Sphaeriales.

Gäumann (7: 209) places most of Theissen's Pseudosphaeriales in the families of the Myriangiales, but says that they are transition forms to the Sphaeriales through a series with gradually decreasing stroma and more apparent ostiola and with increasing gelatinization of the interthelial tissue. In this regard he agrees with Petrak as stated previously. Further (7: 219), he says that

*Leptosphaeria Doliolum* and *Leptosphaeria acuta* (Moug. & Nestl.) Wint. represent the beginning and end points of a series which leads from the genuine Pseudosphaeriales to the genuine Sphaeriales. Then further on (7: 255), he says that the Sphaeriales were erected as a parallel line to the Hypocreales from which they differ through their dark colored, leathery, hard, or carbonaceous perithecia, but recent examinations have shown him that this holds for only a part of the order, while another part of the developmental series is an off-shoot from the Hypocreales. From this he thinks that the Sphaeriales as at present grouped have no right to exist. Then he enumerates the Diaportheen type and the Pseudosphaeriales type as the two types in the Sphaeriales. In other words, he admits that if the fungi of the Pseudosphaeriales type were taken out, then the Sphaeriales would be of one type, and the centrum would then correspond to that found in the Hypocreales.

The mistakes made by the above investigators in the treatment of the Myriangiales were due to a lack of knowledge of the origin of the essential structures in the locule and in the perithecium. The Myriangiales may have arisen, as pointed out, by the reduction of the number of the asci in the locule to one, or these uniascal locular forms may represent the primitive ancestral types of all forms in which the ascal plectenchyma develops in a stroma and is not surrounded by a special wall, such as found in the Phacidiales, Hysteriales, Hemisphaeriales, Perisporiales, Coryneliaceae, Dothideales and Pseudosphaeriales.

### Ordinal Separations in the Pyrenomycetes

In the Pyrenomycetes, the Hypocreales, Sphaeriales, Laboulbeniales, and the Erysiphaceae contain asci in true perithecia. The first two groups are closely related. Both have the Diaportheen type of centrum, true perithecial walls and ostiola. The separation of these two orders in the first place was due to a misconception of the "wall" in the so-called Sphaeriales. The black, carbonaceous "wall" in the Sphaeriales is in reality the stroma. In all the members of the Sphaeriales the true wall is thin, membranaceous, and quite variable in color. Moreover, the stroma is always fleshy when young, becoming woody, leathery,

or carbonaceous when old. Whenever a fungus of the type of *Rosellinia* is found with a relatively thin stromatic layer on the outside of the wall, it is impossible in the present system of classification to know whether to place it in *Rosellinia*, *Sordaria*, or *Melanospora*. In the texture of the stroma *Balansia*, *Myriogenospora*, *Ophiodothis*, *Hypocrella* and *Claviceps*, all in the Hypocreales, exhibit a series that definitely connect the Hypocreales with the Sphaeriales. All have the same type of ascus and ascospore, all have true perithecial wall, but the texture of the stroma varies from hard and carbonaceous to fleshy, and from black on the outside to colored. In view of these facts it seems that the color and texture of the stroma and perithecial walls are of doubtful value in separating these two orders. Therefore, it is suggested that the two be merged as one order characterized by the possession of a true perithecial wall and ostiolum.

The Erysiphaceae have a true perithecial wall, but the lack of an ostiolum should be sufficient to place them in a distinct order.

The membrane in the Laboulbeniales is apparently a true perithecial wall, but the lack of a well-developed mycelium is sufficient to maintain them in a distinct order.

The Myriangiales, Pseudosphaeriales, Perisporiaceae, Coryneliaceae, and Dothideales all belong in one group distinguished by the absence of a true perithecial wall and by the asci being borne in locules in the stroma. They cannot be said to have a perithecium and are, therefore, not Pyrenomycetes.

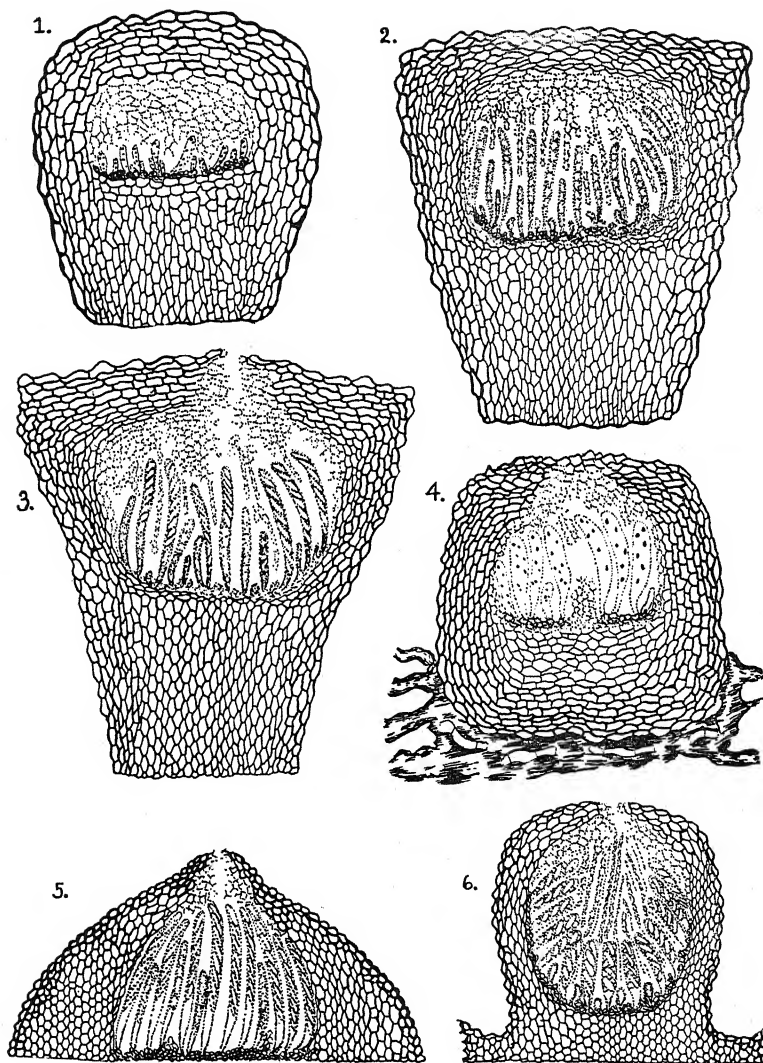
The Erysiphaceae, Hypocreales, Sphaeriales and Laboulbeniales are sharply separated from the above group by the asci being borne in perithecia, and so are true Pyrenomycetes.

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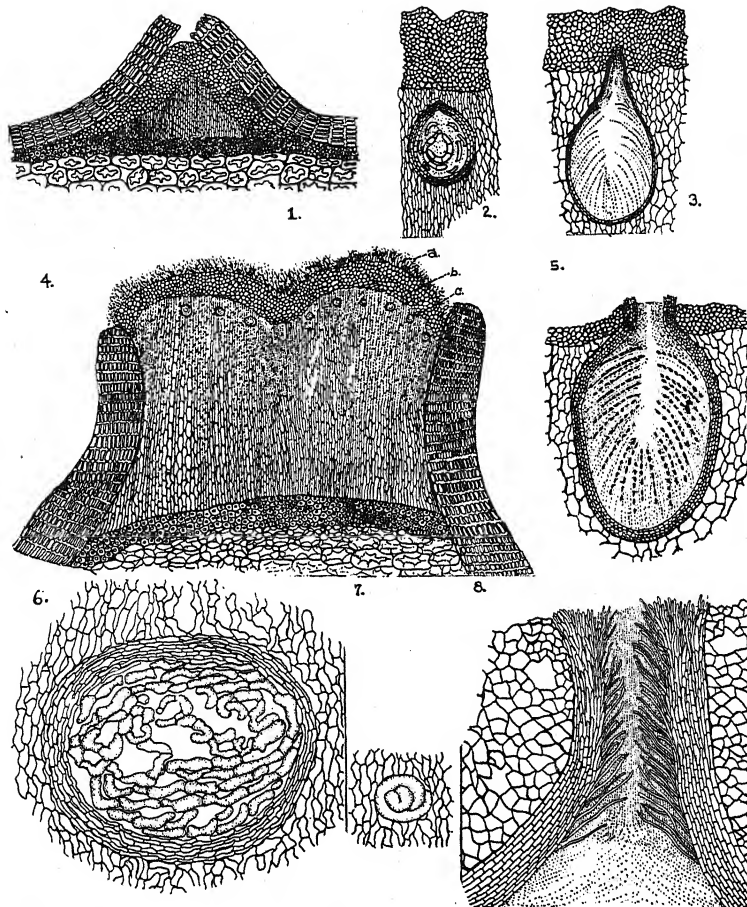
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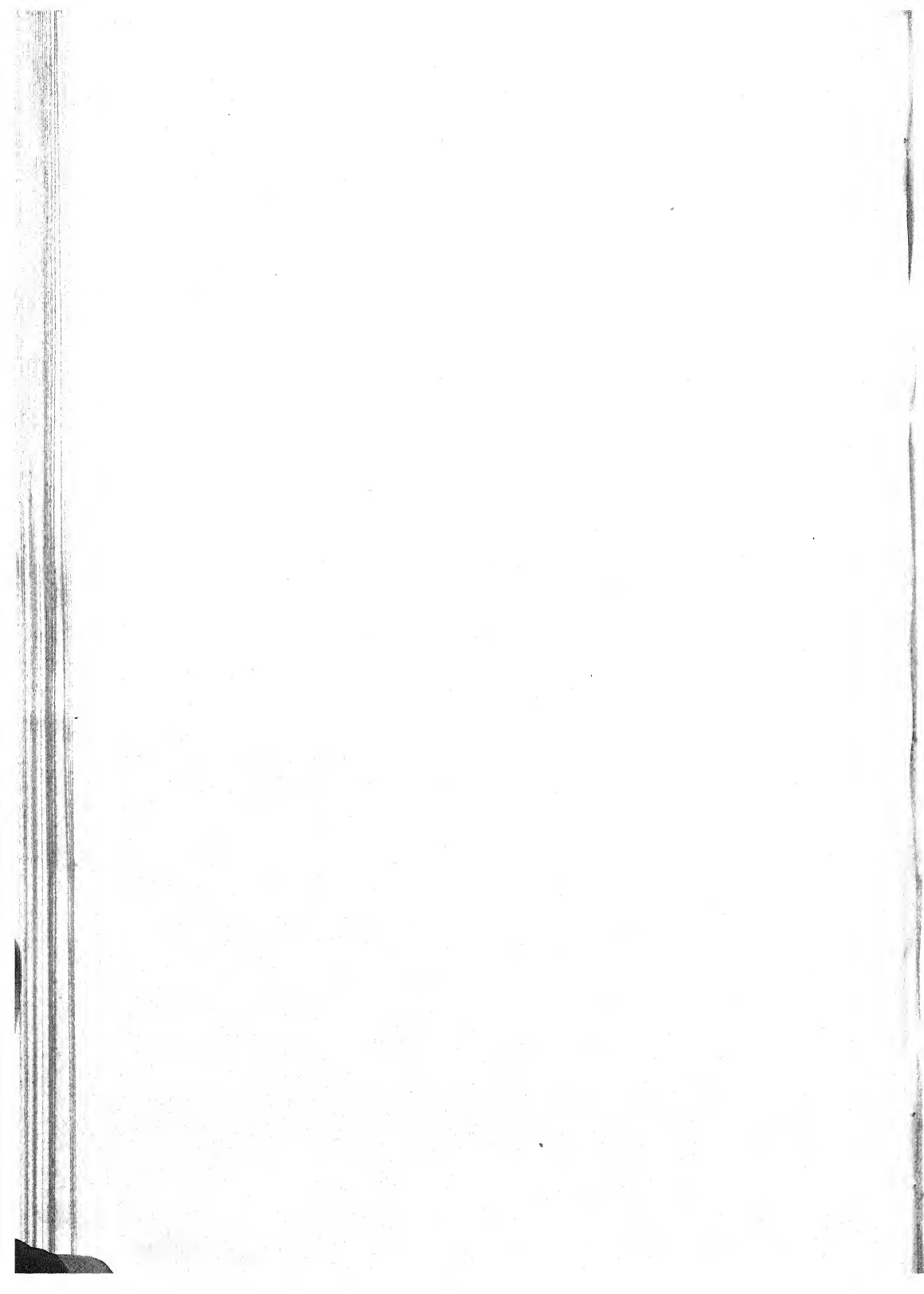


SPHAERIALES





SPHAERIALES



EXPLANATION OF PLATE 21

Fig. 1. *Chaetosphaeria phaeostroma*. Longitudinal section through young stroma showing immature asci growing upward in the pseudoparenchyma.

Fig. 2. *Chaetosphaeria phaeostroma*. Longitudinal section of a more advanced stroma. The pseudoparenchymatous strands are seen to be connected to the upper part of the stroma.

Fig. 3. *Chaetosphaeria phaeostroma*. Longitudinal section through a mature stroma showing the dissolution of the pseudoparenchyma to form an opening.

Fig. 4. *Guignardia Bidwellii*. Longitudinal section through a stroma showing its dothideaceous character.

Fig. 5. *Leptosphaeria Dolium*. Longitudinal section through mature stroma.

Fig. 6. *Dibotryon morbosum*. Longitudinal section through a mature locule. The paraphyses-like threads here are seen to be stromal remnants.

EXPLANATION OF PLATE 22

Development of *Hypoxyton Howeianum* Peck

Fig. 1. Longitudinal section through initial stroma showing the ruptured bark and the ectostroma being pushed upward by the developing entostroma.

Fig. 2. Longitudinal section through a very young perithecium showing the beginning of apical growth of the wall to form the ostiolar canal.

Fig. 3. Longitudinal section through a young perithecium showing the manner in which the perithecial neck penetrates through the ectostroma.

Fig. 4. Longitudinal section through the entire stroma. *a.* conidial layer. *b.* ectostroma. *c.* perithecial initials in the periphery of the entostroma.

Fig. 5. Longitudinal section through a mature perithecium.

Fig. 6. An enlarged view of a longitudinal section of a young perithecium. The entire wall completely shuts out the pseudoparenchyma of the stroma, and encloses only Woronin segments.

Fig. 7. Longitudinal section through an archicarp from which the perithecium, including its content, arises.

Fig. 8. Longitudinal section through the perithecial neck showing paraphyses lining the ostiolar canal.

## STUDIES IN TROPICAL ASCOMYCETES—V. SPECIES OF PHYLLACHORA.

FRED J. SEAVER

(WITH PLATES 23-28)

The genus *Phyllachora* includes a group of parasitic Ascomycetes familiar to the mycologist in the northern regions mainly on grasses and sedges. In connection with our work in tropical islands, the writer has been impressed by the number of species of *Phyllachora* (using the name in a broad sense) which occur there on dicotyledonous hosts. Some of them appear to be undescribed while a number of species from the West Indies have been recently described by F. L. Stevens, C. E. Chardon, and other mycologists who have worked in the islands. Many, however, are very poorly known and an attempt is being made at this time to bring these together and publish them with brief diagnoses and illustrations.

In illustrating the species we have tried to do this in such a way as to show the character of the leaf-spots and at the same time give some idea of the size and form of the spores. In order to accomplish these results the leaves themselves have been properly mounted and the drawings of the spores made to accompany them. These combinations have been photographed together and the results reproduced in the accompanying plates. The drawings of the spores are made with the aid of the camera lucida but some allowance should be made for the sizes indicated in view of the fact that it is difficult to be sure that the spores are mature. This fact probably accounts for some of the discrepancies in the spore measurements as given by different authors.

The present paper includes only a part of the species reported from tropical America and it is expected that a later paper may follow with similar arrangement.

PHYLLACHORA ACACIAE P. Henn. Hedwigia 33: 233. 1894.

*Phyllachora texana* Tharp., Mycologia 9: 118. 1917.

Stromata gregarious and quite evenly scattered over the leaflets, varying in size from minute punctiform dots to more than 1 mm. in diameter, visible on both sides of the leaflets, occasionally several confluent, black and shining, the surrounding tissues reddish-brown; loculi few (usually only one or two) to each stroma, opening on the under side of the leaflet or on both; asci about  $50-80 \times 10-14 \mu$ , 8-spored; spores 1-seriate or irregularly crowded, ellipsoid or subfusoid,  $4-5 \times 12-18 \mu$ ; paraphyses present delicate. (PLATE 27, F. 4.)

On *Acacia acutifera* Benth., Bahama.

*Acacia amentacea* DC., Mexico.

*Acacia coriophylla* Benth., Bahama.

*Acacia unijuga* Rose, Mexico.

*Acacia Wrightii* Benth., Texas.

*Vachellia Farnesiana* (L.) W. & A., Porto Rico, Cuba.

TYPE LOCALITY: Ecuador on *Acacia Farnesiana*.

DISTRIBUTION: Texas to Florida and West Indies; also in South America.

***Phyllachora amyridicola* sp. nov.**

Stromata scattered or more or less aggregate on the upper side of the leaf but also visible on the under surface, very small, not exceeding .5 mm. in diameter, prominent on the upper side of the leaf; loculi few to each stroma, the necks of the ostioli prominent; asci clavate, 8-spored; spores irregularly disposed in the ascus, fusiform,  $8 \times 20-25 \mu$ . (PLATE 24, F. 2.)

Type collected on *Amyris Plumieri* DC., Jamaica, Sept. 18, 1907. (Britton 1507.)

DISTRIBUTION: Known only from the type locality.

***Phyllachora Amyridis* sp. nov.**

Stromata few, scattered over the upper surface of the leaf. Faintly visible on the under surface also, circular in form, convex, on the upper surface slightly rough, shining, reaching a diameter of 1 mm.; loculi numerous, closely crowded, the necks of the ostioli roughening the surface of the stroma; asci clavate, 8-spored, the spores 1-seriate or irregularly disposed, broad-ellipsoid, often slightly constricted in the center,  $6 \times 14 \mu$ . (PLATE 24, F. 1.)

Type collected on *Amyris elemifera* L. in Desecheo Island, Porto Rico, Feb. 18-19, 1914. (Britton, Cowell and Hess 1633.)

On *Amyris balsamifera* L., Cuba.

*Amyris elemifera* L., Florida.

DISTRIBUTION: Porto Rico, Cuba; also in Florida.

PHYLLACHORA ATELEIAE Seaver in Britton & Millsp., The Bahama Flora 632. 1920.

Stromata numerous, appearing on either side of the leaf but more conspicuous on the upper side, black, shining, scarcely exceeding a diameter of 1 mm., perithecia few to each stroma, conspicuous; asci clavate, reaching a diameter of 14-16  $\mu$ ; spores fusoid, hyaline, 4-5  $\times$  18-20  $\mu$ . (PLATE 24, F. 4.)

On *Ateleia cubensis* Griseb., Andros, New Providence, Great Exuma, Cuba.

TYPE LOCALITY: Andros, West Indies.

DISTRIBUTION: Andros and Cuba.

PHYLLACHORA BOURRERIAE Stevens & Dalbey, Bot. Gaz. 68: 54. 1919.

Stromata circular and black, abundant, scattered irregularly over the leaf, 1-2 mm. in diameter, equally prominent above and below, occupying the mesophyll; loculi several, globular, about 160  $\mu$  in diameter with a definite wall; asci cylindrical, 8-spored, 85  $\times$  9-12  $\mu$ ; spores fusiform, 6-7  $\times$  12-16  $\mu$ . (PLATE 20, F. 2.)

On *Bourreria succulenta* Jacq., Porto Rico.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico.

ILLUSTRATIONS: Bot. Gaz. 68: pl. 6, f. 3-4.

Phyllachora Brittoniana (Chardon) comb. nov.

*Catacauma Brittoniana* Chardon, Mycologia 19: 298. 1927.

Spots large, yellowish, 10 to 15 mm. in diameter, very conspicuous on the under surface of the leaf, scarcely so on the upper surface, possessing many confluent stromata; stromata black, not shiny, 2-5 mm. in diameter or even more through the coalescence of various individuals, largely raised and very pronounced on the under surface, sometimes, but not always, following the veins of the leaf, distinctly situated between the epidermis and the mesophyll; loculi several, 2-7 in each stroma, flattened, or angular through lateral pressure; asci cylindrical-



clavate, 8-spored; spores navicular, 1-seriate above, 2-seriate in the main body of the ascus, continuous, hyaline,  $4-5 \times 15-18 \mu$ , possessing one or several oil drops; paraphyses present, profuse. (PLATE 25, F. 6.)

On *Ficus subscabrida* Warb.

TYPE LOCALITY: Isle of Pines.

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Mycologia 19: pl. 27, f. 1.

A fine collection of this species was made by Dr. and Mrs. Britton and Mr. Percy Wilson on the Isle of Pines in 1916, No. 15472. Several years ago the writer undertook the task of illustrating the species *Phyllachora* of the tropics and this was included as an undescribed species. In the meantime, Mr. Chardon described the species and illustrated it as indicated above. It is quite fitting that it should have been dedicated to Dr. N. L. Britton who originally collected the fungus.

PHYLLACHORA CANAFISTULAE Stevens & Dalbey, Bot. Gaz. 68: 55. 1919.

Stromata thickly and rather evenly scattered over the leaf, visible on either side but more conspicuous on the upper, convex and roughened by the protruding necks of the ostiola, shining or with a dull gloss, reaching a diameter of 2-5 mm., irregularly rounded; loculi numerous, opening on the upper surface of the leaf; asci clavate, 8-spored; spores partially 7-seriate, ellipsoid but usually attenuated below,  $6-8 \times 13-16 \mu$ . (PLATE 26, F. 3.)

On *Cassia Fistula* L.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico.

ILLUSTRATIONS: Bot. Gaz. 68: pl. 6, f. 5-6.

*Phyllachora conspicua* (Chardon) Seaver, comb. nov.

*Trabutia conspicua* Chardon, Mycologia 19: 296. 1927.

Stromata thickly scattered over the surface of the leaf but visible on the upper side only, forming irregular patches 1 cm. or more in diameter radiating from a central point in zigzag irregular branching lines about 1 mm. in diameter, smooth and shining; loculi numerous and slightly conspicuous; asci clavate, 8-spored; spores narrow-ellipsoid,  $6-7 \times 20-24 \mu$ . (PLATE 26, F. 2.)

On *Capparis Grisebachii* Eichl.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico; Cuba.

ILLUSTRATIONS: Mycologia 19: *pl.* 27, *f.* 4.

PHYLLACHORA DRYPETICOLA Stevens & Dalbey, Bot. Gaz. 68: 55.  
1919.

Stromata scattered over part of the leaf, equally visible on either side, dull, about 1–2 mm. in diameter; loculi several to each stroma, the necks of the ostiola strongly protruding; asci cylindrical to clavate, 8-spored; spores large, fusoid, 1-seriate to 2-seriate,  $4-7 \times 17-22 \mu$ . (PLATE 27, F. 1, 5.)

On *Drypetes glauca* Vahl., Porto Rico.

*Drypetes lateriflora* (Sw.) Krug & Urban, Florida, Jamaica.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico, Jamaica; also in Florida.

PHYLLACHORA ENGLERI Speg. Anal. Soc. Ci. Argent. 19: 96.  
1885.

*Phyllachora Philodendronis* Pat. Bull. Soc. Myc. Fr. 8: 134.  
1892.

Spots indeterminate, only slightly pale or not at all; stromata densely gregarious, slightly lenticular, visible on both sides of the leaf, reaching a diameter of 3–5 mm., circular or subcircular in form, smooth and shining, black; loculi immersed, closely crowded, lenticular-conoid, small,  $120-140 \mu$  in diameter with the ostiola minute, reaching a diameter of  $120-140 \mu$ ; asci cylindric, truncate above, attenuated below, 8-spored; spores 2-seriate, ellipsoid or ellipsoid-navicular, attenuate at both ends, straight or curved, containing two large oil drops,  $4-5 \times 14 \mu$ . (PLATE 23, F. 1.)

On *Philodendron* sp., Ecuador, Guatemala.

*Anthurium acaule* Schott, Bolivia.

*Anthurium dominicense* Schott, Porto Rico.

*Anthurium Holtonianum* Schott, Colombia.

*Anthurium isertianum* Schott, Martinique.

*Anthurium panduratum* Mart., Brazil.

*Anthurium scandens* (Aubl.) Engler, Jamaica, Porto Rico, Bolivia.

*Anthurium venosum* Griseb., Cuba.

TYPE LOCALITY: Paraguay, on *Spathicarpa lanceolata* Engler.

DISTRIBUTION: West Indies; also in Northern South America.

PHYLLACHORA FUSICARPA Seaver in Britton & Millsp. The Bahama Flora 633. 1920.

Stromata rather numerous, often thickly scattered over the leaf, visible on both sides but more conspicuous on the under side, small, ranging from 1-2 mm., several often confluent; perithecial cavities few to each stroma, opening on the under side of the leaf; asci clavate, 8-spored; spores fusiform or approaching fusiform, slightly unsymmetrical, about  $6 \times 25-30 \mu$ . (PLATE 23, F. 3.)

On *Duranta repens* L., Bahama, Porto Rico.

TYPE LOCALITY: New Providence.

DISTRIBUTION: Porto Rico, Bahama.

PHYLLACHORA GRATISSIMA Rehm, Hedwigia 31: 306. 1892.

Stromata epiphyllous, for the most part occurring on yellowish spots which reach nearly 1 cm. in diameter, usually several stromata on each spot, the stroma convex, rough, black, shining, usually 1-2 mm. in diameter but often several confluent, visible on the under side of the leaf, yellowish spots; perithecia immersed, the ostiola rather prominent, comparatively few to each stroma; asci clavate, about  $20 \times 100-110 \mu$ , 8-spored; spores very large, ellipsoid, unequal sided, granular within, hyaline or slightly yellowish,  $12-14 \times 20-25 \mu$ . (PLATE 24, F. 3.)

On Louraceae.

*Persea Persea* (L.) Cockerell (*Persea gratissima* L.), Porto Rico, Jamaica.

TYPE LOCALITY: Ecuador.

DISTRIBUTION: West Indies; also in South America.

EXSICCATI: Rehm, Ascom. 1974.

PHYLLACHORA INCLUSA (Berk. & Curt.) Sacc. Syll. Fung. 3: 599. 1883.

*Dolhidea inclusa* Berk. & Curt. Proc. Am. Acad. Arts & Sci. 4: 129. 1860.

Stromata thickly scattered over the surface of the leaf, often about equally visible on either side, fairly prominent, small, not exceeding .5 mm. in diameter, subcircular in form, surface minutely roughened, dull; loculi one or few to each stroma, not conspicuous; asci clavate, 8-spored; spores fusoid,  $10 \times 15-20 \mu$ . (PLATE 28, F. 5, type.)

On *Jacquinia Berterii* Spreng., Porto Rico.

TYPE LOCALITY: Nicaragua, on *Jacquinia* sp.

DISTRIBUTION: Porto Rico; also in Nicaragua.

In the type collection on a thin-leaved unnamed species of *Jacquinia* the stromata are about equally visible on either side of the leaf, while in the Porto Rican specimen on a thick-leaved species of *Jacquinia* they are scarcely visible on the under side.

PHYLLACHORA LATHYRI (Lév.) Theiss. & Syd. Ann. Myc. 13: 501. 1915.

*Dothidea Lathyri* Lév. Demidoff Voyage 2: 106. 1915.

?*Mazzantia fennica* Lind, Ann. Myc. 13: 22. 1915.

Stromata numerous and scattered over the upper surface of the leaf but visible on the under surface as well, either subcircular or irregular in form, convex, on the upper side uneven and dull, reaching a diameter of 1 mm.; loculi numerous, rather close together; asci cylindrical or clavate, 8-spored; spores 1-seriate, narrow-ellipsoid, more narrowed at the lower end,  $6-8 \times 13-17 \mu$ . (PLATE 23, F. 5.)

On *Bradburya virginiana* (L.) Kuntze.

*Galactia* sp.

TYPE LOCALITY:

DISTRIBUTION: Porto Rico; also in Europe and Asiatic Russia.

PHYLLACHORA MAYEPEAE Stevens & Dalbey, Bot. Gaz. 58: 56. 1919.

Spots irregularly circular, indefinite without border, tan or yellow, shading to normal green, 3-15 mm. in diameter, bearing numerous (5-50) circular, black, punctiform stromata which are visible above and below, about 1 mm. in diameter, occupying the mesophyll, each stroma containing one perithecial cavity; asci  $18-27 \times 58-85 \mu$ , 8-spored; spores  $7-8 \times 18-20 \mu$ , hyaline. (PLATE 27, F. 2.)

On *Mayepea domingensis* (Lam.) Krug & Urban.

TYPE LOCALITY: Maricao, Porto Rico.

DISTRIBUTION: Porto Rico.

PHYLLACHORA MYRCIAE (Lév.) Sacc. Syll. Fung. 2: 597. 1883.

*Dothidea Myrciae* Lév. Ann. Sci. Nat. III. 5: 264. 1846.

*Catacauma Myrciae* Theiss. & Syd. Ann. Myc. 13: 393. 1915.

Stromata gregarious, one to a dozen on each leaf, occurring on

the under side of the leaf and not visible on the upper side, circular or subcircular in form, reaching a diameter of 2-3 mm., smooth, shining; perithecial cavities disposed in a circle around a central point, more or less confluent; asci broad, clavate,  $20 \times 60-75 \mu$ , 8-spored; spores acute at either end, strongly curved so as to appear half-moon shaped, measuring  $18-20 \mu$  from horn to horn, with a thickness of  $8 \mu$ , occasionally nearly straight (apparently when young). (PLATE 23, F. 4.)

On *Myrcia paniculata* (Jacq.) Krug & Urban, Porto Rico, Virgin Islands.

TYPE LOCALITY: Brazil, on *Myrcia* sp.

DISTRIBUTION: Porto Rico, Virgin Islands; also in South America.

Theissen and Sydow who have examined the original specimen of this species state "Asken und Sporen waren nicht mehr vorhanden." The spores were described as subarcuate, while the spores in our specimen are more than subarcuate. They are decidedly lunulate. The specimen however doubtless belongs here.

PHYLLACHORA NECTANDRAE Stevens & Dalbey, Bot. Gaz. 68: 57. 1919.

Stromata situated on brown spots on the upper side of the leaf, but visible on the under side, scattered or more rarely confluent, black, shining, reaching a diameter of 1-4 mm.; loculi single or few to each stroma; asci cylindrical or clavate, 8-spored; spores ellipsoid,  $5 \times 14 \mu$ . (PLATE 28, F. 2.)

On *Nectandra patens* (Sw.) Griseb.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico.

PHYLLACHORA NITENS Garman, Mycologia 7: 339. 1915.

Stroma forming a large, black, shining spot often covering an area of several cm., appearing on both sides of the leaf; perithecial cavities numerous but not prominent; asci clavate, 8-spored,  $12-16 \times 100-110 \mu$ ; spores ovoid, acute at one end,  $8 \times 12 \mu$ , often with an appendage  $6-8 \mu$  long. (PLATE 27, F. 4.)

On *Schlegelia brachyantha* Griseb., Porto Rico.

*Schlegelia axillaris* Griseb., Dominica.

TYPE LOCALITY: Maricao, Porto Rico, on *Schlegelia brachiata*.

DISTRIBUTION: Porto Rico.

PHYLLACHORA OCOTEICOLA Stevens & Dalbey, Bot. Gaz. 68: 57. 1919.

Stromata sparingly scattered over the leaf, about equally visible on either side, not prominent on either, irregularly rounded, subshining, reaching a diameter of 2-4 mm; loculi several to each stroma, not very prominent; asci cylindrical to clavate, 8-spored; spores partially 2-seriate, ellipsoid, or fusoid,  $7-8 \times 17-20 \mu$ . (PLATE 27, F. 3; PLATE 28, F. 3.)

On *Ocotea leucoxylon* (Sw.) Mez.

TYPE LOCALITY: Porto Rico.

DISTRIBUTION: Porto Rico.

*Phyllachora Pennellii* sp. nov.

Stromata distributed over the surface of the leaf and about equally visible on either side, forming beautiful patterns on the substratum like Chinese writing or hieroglyphics, elongated and radiately branched, the branches reaching a length of 1-2 cm. and a diameter of 1-2 mm., dull; loculi numerous and roughening the surface of the stroma; asci clavate, 8-spored; spores ellipsoid, 1-seriate or 2-seriate, about  $6-7 \times 12-14 \mu$ , slightly yellowish. (PLATE 26, F. 1.)

Type collected on leaf of some unknown host by Dr. F. W. Pennell, in Colombia, altitude 100-300 m., March 7-10, 1918.

DISTRIBUTION: Known only from the type collection.

This species with its unique and striking pattern was collected by Dr. Pennell in the lowland tropical forest of the Rio Sinu, Northern Colombia. Unfortunately we are at present unable to name the host. Dr. Pennell writes: "At the time this was collected the dry season had already begun and there were many leaves on the forest floor. It would likely have been difficult even then to have procured a specimen of tree as in dense forests it is not easy to say from which tree a dead leaf has fallen." So we will necessarily have to wait for further information on this point. There should be no difficulty in recognizing the fungus should it be encountered again.

PHYLLACHORA PERIBEBUYENSIS Speg. Anal. Soc. Ci. Argent. 19: 244. 1886.

*Auerswaldia Miconiae* P. Henn. Hedwigia 43: 253. 1904.

*Dothidina peribebuyensis* Chardon, Mycologia 13: 289. 1922.

Stromata numerous, thickly scattered over the under surface of the leaf and slightly visible on the other side, reaching a diameter of 1-2 mm., rough and dull in appearance; loculi subimmersed but prominent; asci cylindrical to clavate, 8-spored; spores 1-seriate or becoming partially 2-seriate, oblique or irregularly disposed, ellipsoid, often slightly constricted in the center. (PLATE 23, F. 7.)

On *Heterotrichum cymosum* (Wendl.) Urban.

*Miconia laevigata* (L.) DC.

*Miconia prasina* (Sw.) DC.

*Miconia Sintenisii* Cogn.

*Tetrazygia elaeagnoides* (Sw.) DC.

TYPE LOCALITY: South America.

DISTRIBUTION: Porto Rico; continental South America.

PHYLLACHORA PHASEOLI (P. Henn.) Theiss. & Syd. Ann. Myc.  
13: 507. 1915.

*Physalospora Phaseoli* P. Henn. Hedwigia 43: 368. 1904.

*Hyponectria Phaseoli* Stevens, Bot. Gaz. 70: 401. 1920.

Stromata numerous, thickly gregarious on yellowish spots about 1 cm. in diameter, visible on both sides of the leaf but opening on the upper side, conspicuous, black, shining; asci cylindric or subcylindric, 8-spored; spores 1-seriate, broad-ellipsoid or subglobose, 8-10  $\times$  12  $\mu$ . (PLATE 25, F. 2.)

On *Vigna vexillata* (L.) A. Rich.

TYPE LOCALITY: South America, on *Phaseolus* sp.

DISTRIBUTION: Porto Rico, Grenada; also in South America.

PHYLLACHORA RANDIAE Rehm, Hedwigia 36: 371. 1897.

*Trabutia Randiae* Theiss. & Syd. Ann. Myc. 13: 351. 1915.

Stromata on the upper side of the leaves, small, but forming often a number of confluent patches up to 5 mm. in diameter, the patches presenting a convoluted surface, smooth and shining; loculi few to each stroma, strongly protruding; asci clavate, 8-spored; spores rather broadly ellipsoid. (PLATE 28, F. 1.)

On *Randia pubescens* Ruiz & Pav., Bolivia.

*Randia mitis* L., Porto Rico; Hayti; Trinidad.

TYPE LOCALITY: Bolivia.

DISTRIBUTION: West Indies; also in South America.

PHYLLACHORA SECURIDACAE P. Henn. Hedwigia 43: 251. 1904.

Stromata sparingly scattered over the leaflets and about equally visible on either side, very small, not exceeding .5 mm. in diameter, slightly convex and dull; loculi few to each stroma, not conspicuous; spores rather small, fusoid or fusiform, about  $5-6 \times 15 \mu$ . (PLATE 25, F. 1.)

On *Elsota virgata* (Sw.) Kuntze (*Securidaca virgata* Sw.).

TYPE LOCALITY: South America.

DISTRIBUTION: Porto Rico; also in South America.

PHYLLACHORA SIMPLEX Starb. Arkiv. Bot. 5<sup>15</sup>: 14. 1905.

Stromata numerous and scattered over the upper surface of the leaf but also visible on the under side, very small, only about 3 mm. in diameter, occasionally several confluent along the midrib of the leaf, usually nearly circular, conspicuous and shining; loculi few, 1-3 in each stroma, the necks of the ostiola prominent; asci club-shaped, 8-spored; spores bunched or irregularly disposed in the ascus, narrow-ellipsoid or more attenuated at one end. (PLATE 23, F. 6.)

On *Coccolobis laurifolia* Jacq.

TYPE LOCALITY: Paraguay.

DISTRIBUTION: Porto Rico; continental South America.

PHYLLACHORA TRAGIAE (Berk. & Curt.) Sacc. Syll. Fung. 2: 601. 1883.

*Dothidea Tragiae* Berk. & Curt. Jour. Acad. Nat. Sci. II. 2: 288. 1853.

Spots scattered, small, black, shining, surrounded by a very narrow whitish area, reaching a diameter of 1 mm., visible on both sides but more distinct on the under surface; perithecial cavities closely crowded, usually about 6-10 to each stroma; asci clavate, 8-spored,  $18-20 \times 90 \mu$ ; spores ellipsoid, filled with small granules,  $7-8 \times 14-16 \mu$ . (PLATE 25, F. 3.)

On *Croton lucidus* L., Porto Rico.

*Croton flavens* L., Porto Rico.

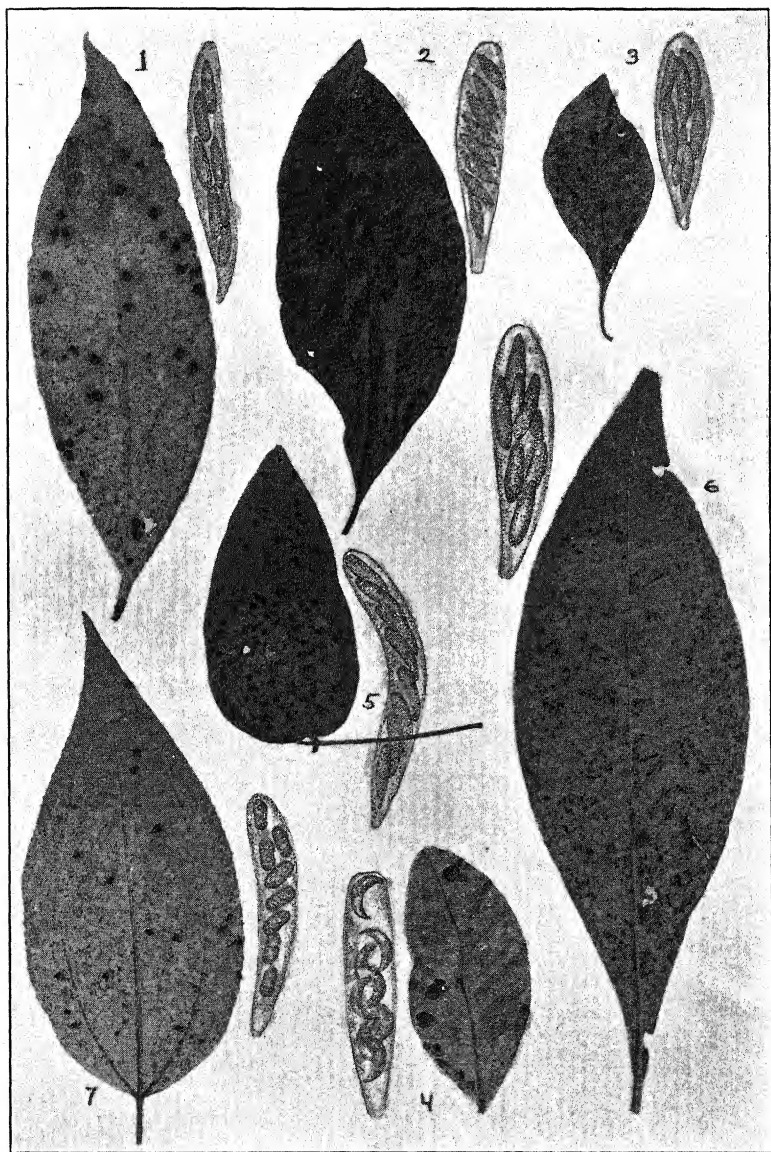
TYPE LOCALITY: South America.

DISTRIBUTION: Porto Rico; also South America and Panama.

PHYLLACHORA ZANTHOXYLI Winter; Rab.-Wint. Fungi Eur. 3558. 1886.

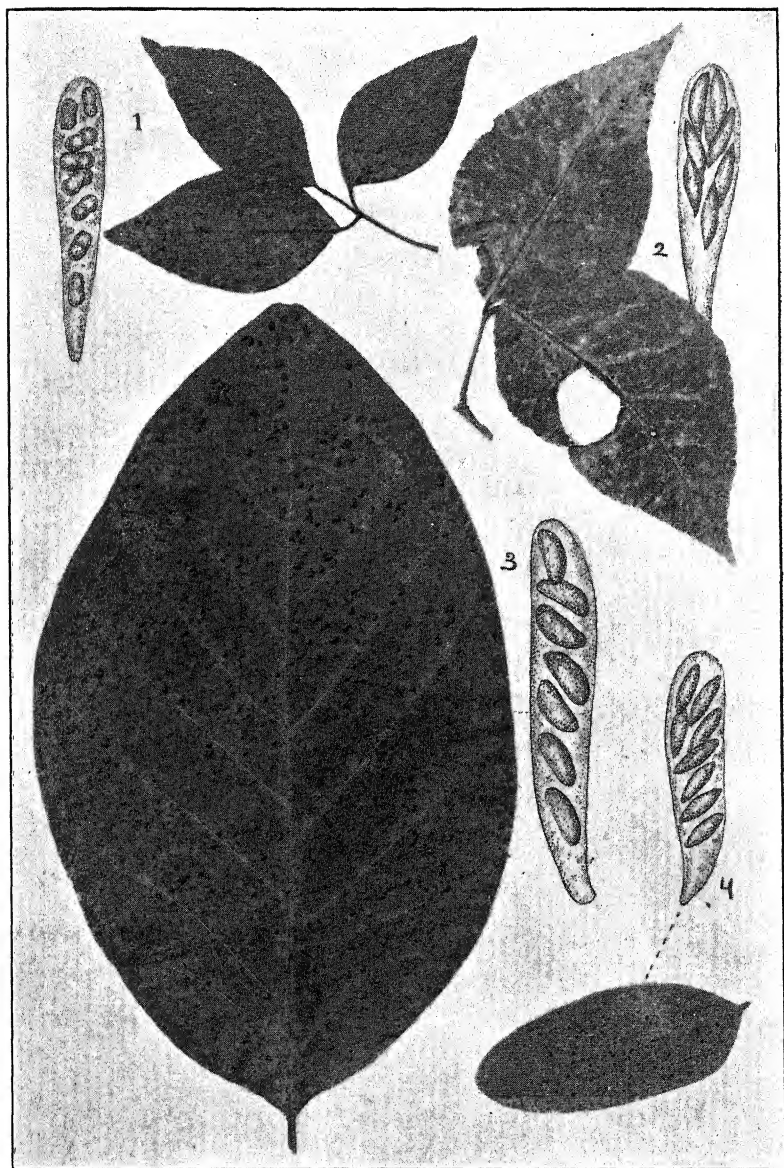
*Trabutia Zanthoxyli* Chardon; Seaver & Chardon, Sci. Surv. Porto Rico 8: 55. 1926.





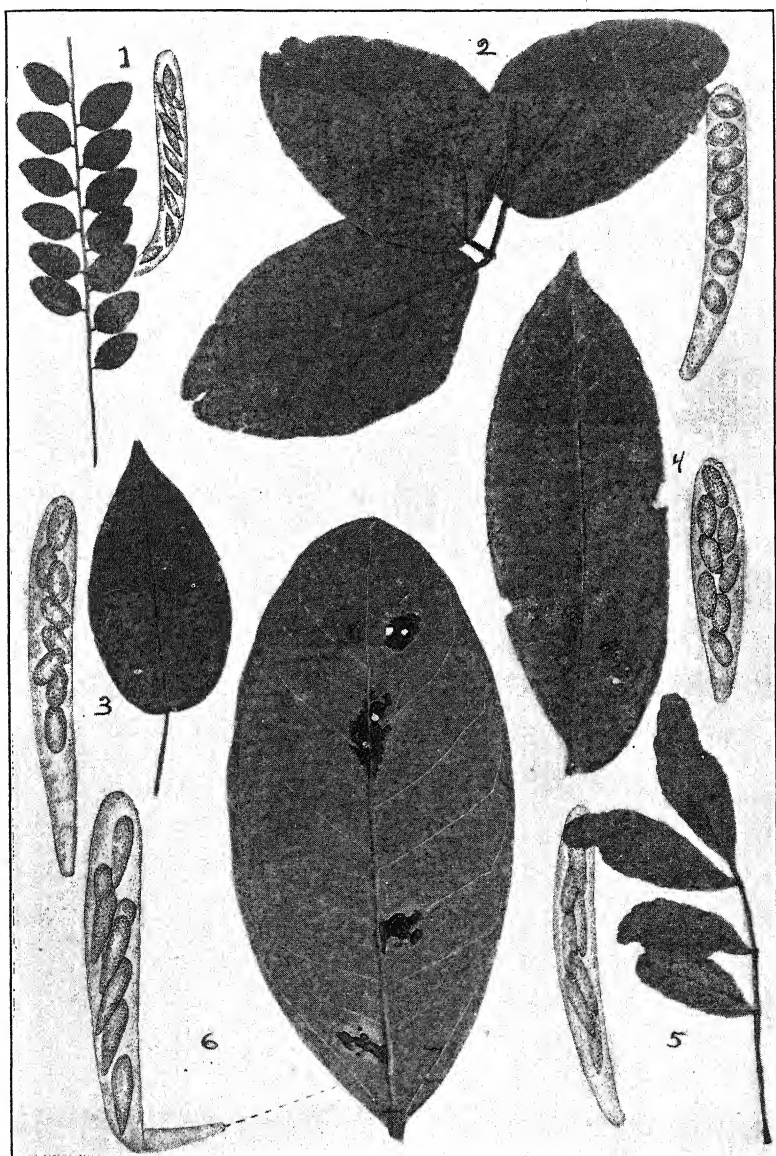
SPECIES OF PHYLLACHORA





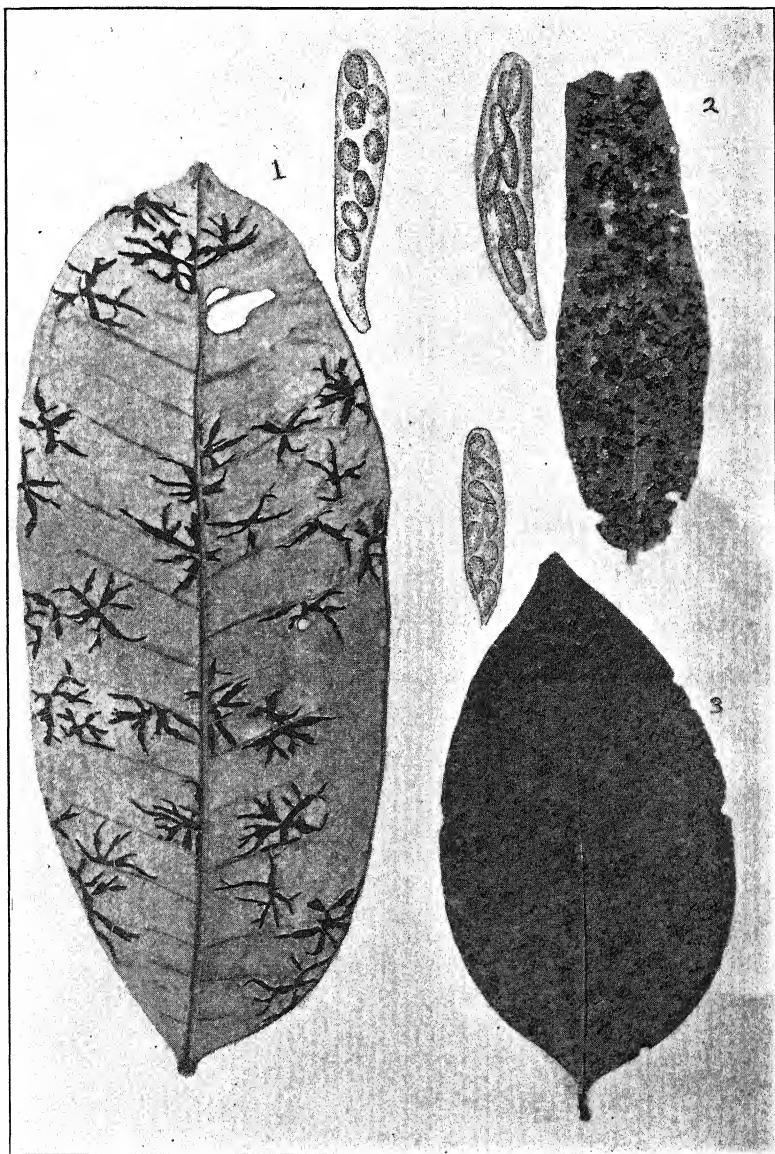
SPECIES OF PHYLLACHORA





SPECIES OF PHYLLACHORA

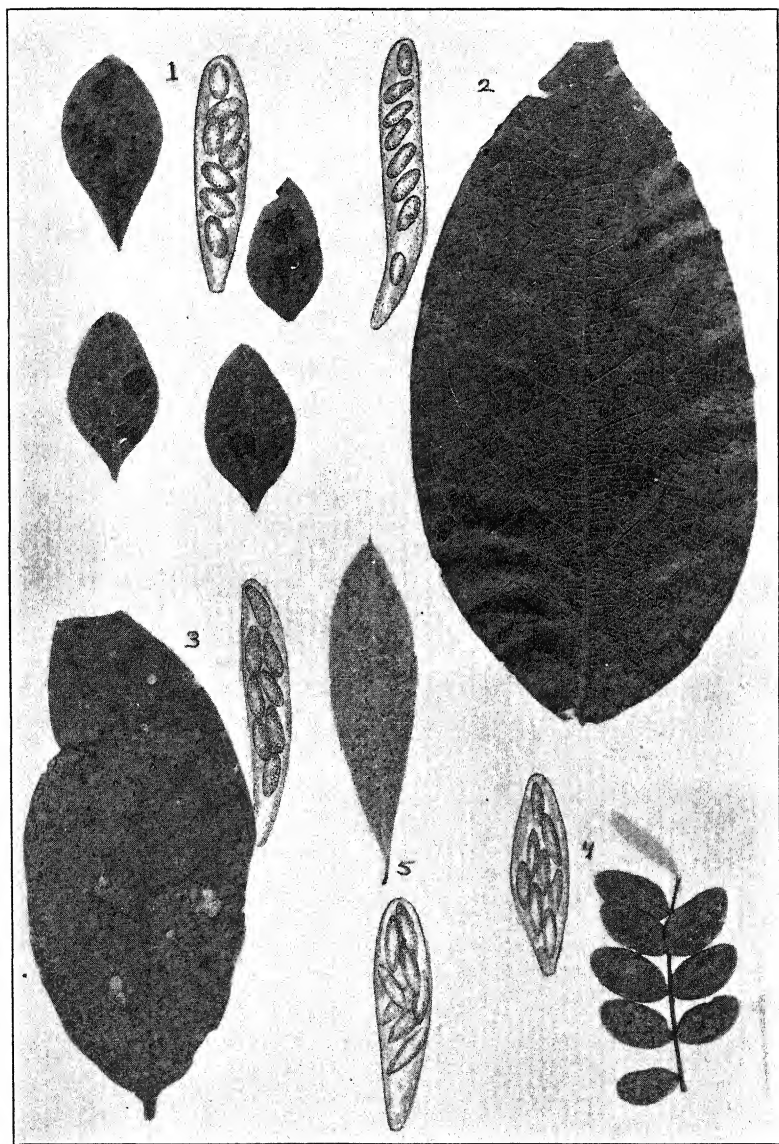




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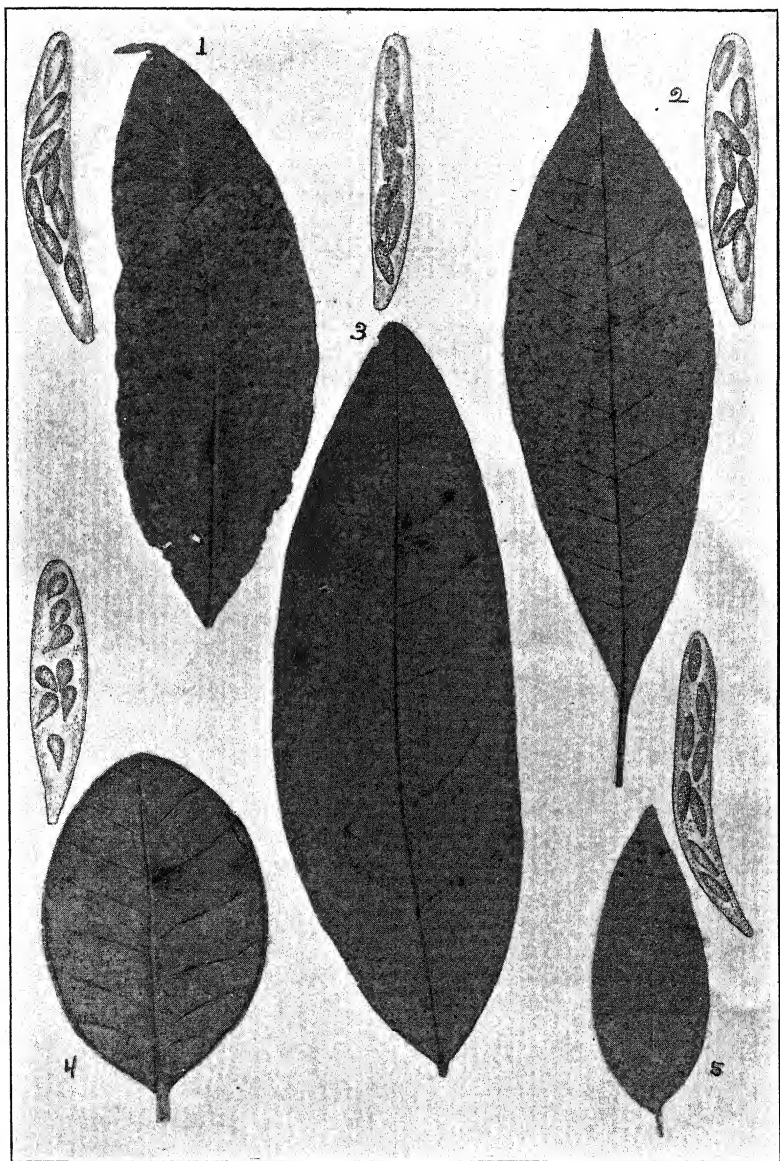




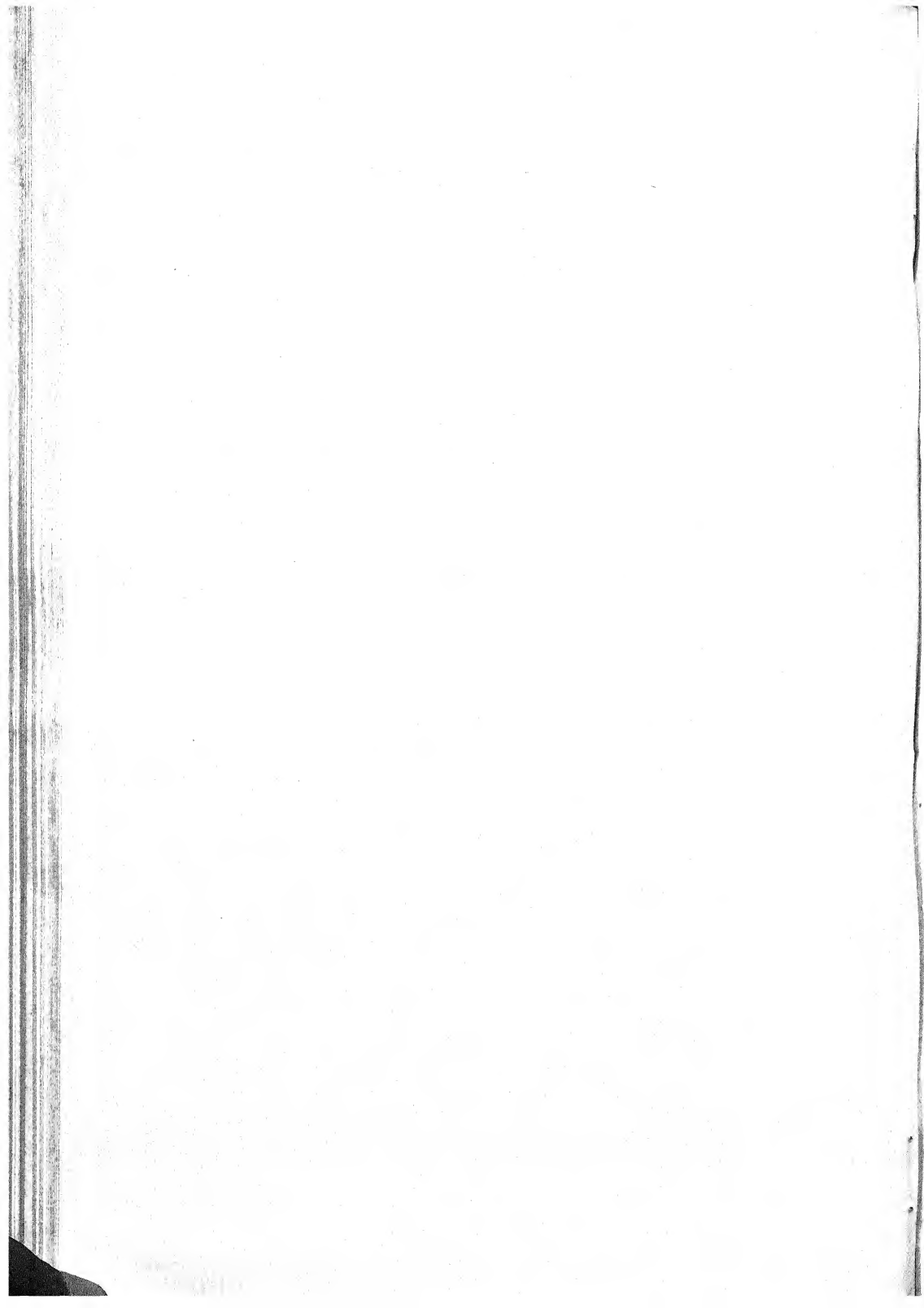


SPECIES OF PHYLLACHORA





SPECIES OF PHYLLACHORA



Stromata occurring on the upper side of the leaf, often on discolored spots, small, rounded or fused together in irregular patches, usually not over 1 mm. in diameter, conspicuous, rather dull; loculi few to each stroma, the ostiola conspicuous; asci subcylindric, 8-spored; spores 1-seriate or partially 2-seriate, ellipsoid, about  $6-7 \times 12-18 \mu$ . (PLATE 25, F. 4.)

On *Zanthoxylum martinicense* (Lam.) DC.

TYPE LOCALITY: South America.

DISTRIBUTION: Porto Rico; also in South America.

***Phyllachora zanthoxylicola* sp. nov.**

Stromata scattered, usually only two or three on a leaflet, nearly circular in form, visible on both sides of the leaf, black, dull, about 1 mm. in diameter; loculi several to each stroma, the necks of the ostiola visible but not prominent; asci clavate, 8-spored; spores usually 2-seriate or irregularly disposed, fusoid, very long, about  $7-8 \times 30 \mu$ . (PLATE 25, F. 5.)

Type collected on *Zanthoxylum insulare* Rose in Jamaica. (E. G. Britton 443.)

DISTRIBUTION: Known only from the type locality.

THE NEW YORK BOTANICAL GARDEN

## UNISEXUAL CONIDIA FROM BISEXUAL MYCELIA

B. O. DODGE

(WITH 1 TEXT FIGURE)

The opinion prevails, in the absence of adequate proof to the contrary, that a heterothallic or a homothallic species of the Mucoraceae remains fixed so far as this particular characteristic is concerned. Only unisexual spores are developed in a sporangium of *Phycomyces nitens*. Only bisexual spores are formed in a sporangium of *Sporodinia grandis*. Species of the ascomycete genus *Neurospora* produce conidia of the *Monilia sitophila* type.<sup>1</sup> *Neurospora sitophila* and *N. crassa* are heterothallic; therefore, in order to obtain perithecia it becomes necessary to mate in culture a pair of haplonts, "A" and "B," which may be conceived as being of opposite sex. It is customary to obtain such unisexual mycelia by germinating ascospores.

A very interesting question arises when one considers the nature of the conidia produced in a mixed culture containing two mycelia of reciprocal sexuality. Clearly each mycelium produces very early its own unisexual conidia. One naturally assumes that in such a culture there would occur hyphal fusions particularly between branches which are of opposite sex. In that case, do such fusion cells bud out and finally produce bisexual conidia? *Neurospora tetrasperma* is ordinarily homothallic. A monosporous mycelium produces perithecia under proper cultural conditions. By selecting an abnormally small ascospore to be found in almost any spore print, one obtains a mycelium which develops perithecia only when mated with another haplont of the opposite sex. In other words, by properly selecting ascospores one can secure heterothallic strains of a species which is commonly homothallic. The writer has recently shown why the

<sup>1</sup> Shear, C. L. & Dodge, B. O. 1927. Life histories and heterothallism in the red bread-mold fungi of the *Monilia sitophila* group, Jour. Agr. Research 34: 1019-1042, illus.

small ascospores are unisexual.<sup>2</sup> The change from homothallism to heterothallism is merely a temporary one, although the originally isolated unisexual mycelia must continue to be heterothallic. One has only to mate two such mycelia in culture and then germinate one of the ascospores from a four-spored ascus in order to recover a homothallic strain. Since it may be presumed that it is the natural thing for *N. tetrasperma* to produce bisexual conidia, one would expect that if he gave the hyphae of two haplont mycelia of reciprocal sexuality a chance to anastomose, new branches of a secondary or homothallic mycelium would arise, and would then later produce bisexual conidia. This would certainly be more logical than to expect that hyphae of reciprocal haplonts of normally heterothallic species would fuse in culture and then develop secondary mycelia capable of producing bisexual conidia.

Even though it is the natural thing for a homothallic mycelium to produce bisexual spores, does not *Neurospora tetrasperma* sometimes produce abnormal conidia just as it does abnormal ascospores? There are, then, two questions to be answered. First, does a homothallic (bisexual) mycelium ever produce unisexual conidia; and second, do hyphal branches of a unisexual mycelium, which one may call haplont A, anastomose in culture with hyphae of haplont B and this result in the production of bisexual hyphal branches and conidia? Results of culture work presented in this paper show that while the first question can be answered in the affirmative, the second probably must be answered in the negative.

Single ascospore cultures were made by germinating what were judged to be normal homothallic spores of *Neurospora tetrasperma*. Of some 50 such cultures only one failed to produce perithecia and it was discarded. In applying methods for germinating ascospores, as originally worked out by the writer,<sup>3</sup> to these *Monilia* forms whose perfect stages are species of the genus *Neurospora*, it should be borne in mind that the ascospores may be stimulated

<sup>2</sup> Dodge, B. O. 1927. Nuclear phenomena associated with heterothallism and homothallism in the ascomycete *Neurospora*, Jour. Agr. Research 35: 289-305, illus.

<sup>3</sup> Dodge, B. O. 1912. Methods of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torrey Club 39: 139-197, illus.

to germinate by subjecting them to a degree of heat which would not be sufficient to kill the conidia which are also very resistant to heat. The point to be emphasized here is that not only may the heating fail to kill the conidia, but it may actually delay their germination for a considerable length of time so that in selecting a single germinating ascospore one has to guard against the carrying over of a very small ungerminated conidium which would later on, perhaps after three or four days, germinate and involve him in errors.

Using a suspension of conidia obtained from one of the fertile cultures containing homothallic mycelia, plates were poured, and 31 single conidium mycelia were isolated. Of this number 21 produced perithecia. The other 10 produced conidia and numbers of the sterile bodies characteristic of haplont mycelia, but no perithecia. Again selecting at random a second generation culture from among the 21 fertile cultures that had developed ascocarps, another set of poured plates containing conidia from the chosen culture was made. Twelve of the 21 mycelia arising from single conidia which were isolated produced perithecia, and 9 did not. Selecting a culture from among the 12 fertile cultures of the third generation, poured plates were again made of conidia, and 80 additional mycelia originating from single conidia were isolated. Of this number, 61 produced perithecia and 19 developed only the sterile bodies and conidia. A fourth set of 23 single conidium mycelia was obtained in a similar manner. Sixteen cultures matured perithecia and 7 did not. The average production of unisexual conidia by the 155 homothallic mycelia was, in these experiments, 29 per cent, which is certainly a surprisingly high percentage. These results were later checked up by a new set of cultures starting with a single ascospore. The germinating spore was transplanted to cornmeal agar in a petri dish and allowed to grow for about 24 hours during which time it was examined at intervals to determine whether any conidia had been carried over by mistake. The tip end of a hyphal branch which could be traced back directly to the ascospore was then cut off and transferred to agar in a test tube.

By the time the culture was 6 days old perithecia had begun to mature and conidia from the tube were sowed on the surface of



cleared cornmeal agar at 10 A.M. The plates were set in a warm room. About 6 hours later when it was found that some of the conidia were growing, 24 single germinating conidia were isolated and planted on cornmeal agar in tubes. The plates from which the conidia had been obtained were placed in an ice box until 12 M. of the following day. Many conidia which had not germinated the preceding afternoon were now beginning to grow so that 18 additional single conidium cultures were obtained. They were examined 6 days after the last isolations were made. While the source of the two sets of cultures was the same, the results were not at all comparable. Of the 24 cultures constituting the first set, 21 developed perithecia and only 3 or about 11 per cent remained sterile. At the same time perithecia were produced in only 6 of the 18 cultures of the second set. Twelve cultures remained sterile, and two-thirds of the mycelia of the second set were thus proved to be unisexual. The average production of unisexual conidia as shown for the two sets was 37.7 per cent. The experiment was repeated under about the same conditions with practically the same results.

The following suggestion is offered to account for the discrepancy. A large conidium provided with two or more nuclei would contain more food and, being more vigorous, would germinate more quickly, particularly if the nuclei were of opposite sex, than would the smaller conidia. If the same plate were examined 24 hours later, it would be found that the germ tubes from conidia that had germinated the previous day would have been so long and much branched as to make their isolation impracticable. The unisexual conidia being the last to germinate would be the ones chosen the second day, thus accounting for the  $66\frac{2}{3}$  per cent unisexual mycelia obtained in the second set.

By growing or pairing separately 19 of the unisexual mycelia secured in the way described above with strains  $S_6$  and  $S_1$ ,<sup>4</sup> it was found that 12 were sexually like  $S_6$  and 7 were like  $S_1$ . The others were not tested out.

An explanation to account for the development of unisexual

<sup>4</sup> The reader will find a discussion of culture methods employed and an explanation of the terms used here in a paper by the writer on "The production of fertile hybrids in the ascomycete *Neurospora*," Jour. Agr. Research 36: 1-14, 1928. illus.

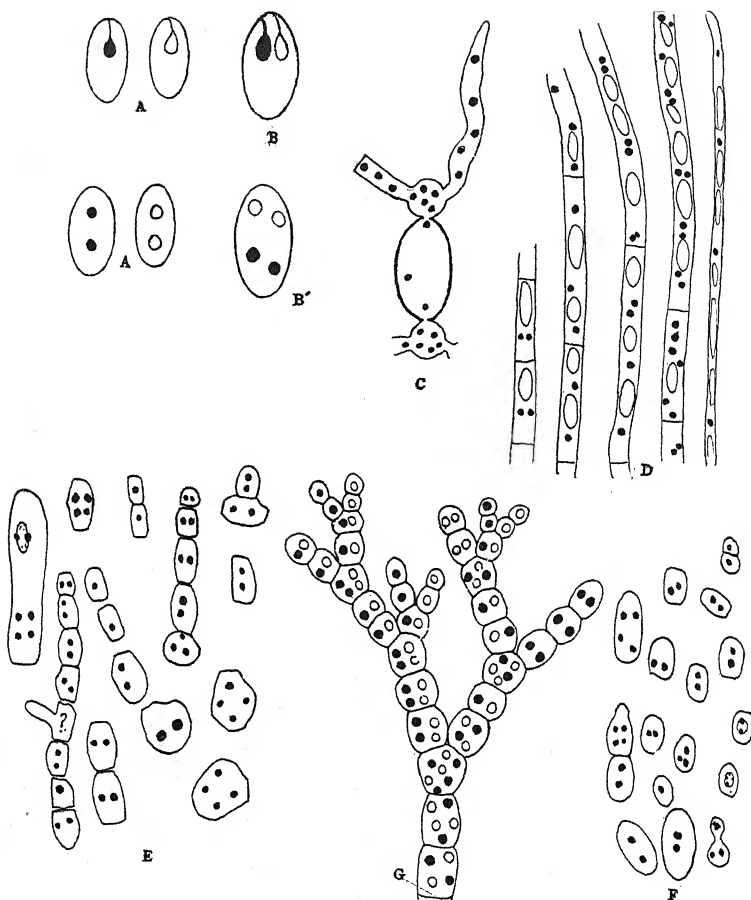


FIG. 1, A to F. Diagram of nuclear condition in cells and spores of *Neurospora tetrasperma*, magnifications not definite; G purely theoretical: A, young unisexual ascospores; A', same spores mature; B, young bisexual spores; B', mature spores; C, germinated ascospore, no cross walls in the young germ tubes; D, young hyphae, position of vacuoles and nuclei shown diagrammatically; E, conidia and fragments of sporogenous hyphae from a homothallic mycelium; F, from unisexual strains showing paired nuclei, a few with a single nucleus; G, diagram suggesting how unisexual conidia might be formed on homothallic mycelia (male nuclei are indicated in black, and female in white); at the right if the nuclear divisions are merely simultaneous or irregular; at the left, binucleate conidia would be bisexual, only uninucleate conidia unisexual.

conidia by homothallic mycelia is not difficult to find. Segregation of the sex factors takes place during nuclear divisions in the ascus, so that, preceding spore formation, the ascus contains four nuclei of each sex. The normal ascospore contains two nuclei of opposite sex at its origin (FIG. 1, *B*). Each of these nuclei divides as the spore matures so that each ascospore finally contains four nuclei (FIG. 1, *B'*). The wall of the spore is rather thick, and the spore does not swell very much as it begins to germinate. Instead, a rather large vesicle is formed by the extrusion of some of the spore contents through the germ pore. Stained preparations show that the vesicle contains several nuclei, sometimes as many as 8 or 10 (FIG. 1, *C*). Germ tubes then grow out from the vesicle in two directions. The cells of the mycelium would naturally contain both kinds of nuclei. Should there be anything corresponding to conjugate nuclear divisions as hyphal branches are developed, each conidium should receive at least one pair of nuclei of opposite sex (FIG. 1, *G*<sup>5</sup>). If the divisions are merely simultaneous, or if they occur irregularly, then by the laws of chance some conidia should be bisexual and others should be unisexual. There is always the possibility that a conidium would be cut off with only a single nucleus and if there had been no nuclear fusions during the development of the mycelium every conidium of this sort would be unisexual.

There is a close correlation between the size of an ascospore of *Neurospora tetrasperma* and the number of nuclei it contains. The small uninucleate, unisexual spores are about 20 to 25  $\mu$  long. This is about the size of the ascospores of *N. sitophila*. Spores that have four nuclei when they are cut out are between 40 and 50  $\mu$  long. Some asci produce only a single giant spore which contains all eight nuclei. If one compares the conidia of *N. sitophila*, which are of course unisexual, with the bisexual conidia of *N. tetrasperma*, he finds that the conidia borne on the unisexual mycelium of the former species are about the same size as the ones borne on the bisexual mycelium of the latter species. Conidia of these forms vary exceedingly in size. Furthermore it is difficult to determine which structures are conidia and which ones are merely the *Oidium*-like fragments of hyphal branches. For this

<sup>5</sup>This figure is not intended to show the order in which conidia are produced.

and other reasons a statistical study of sizes of conidia of different types of mycelia is likely to prove unsatisfactory.

As the writer will be unable to continue this work, he is reporting at this time results obtained in a preliminary study of the cytology of mycelia and conidia which may be suggestive. A glance at material stained in toto with acid fuchsin and iodine green shows that the cells of the homothallic strains ordinarily contain from two to six or eight nuclei, more or less paired as indicated in the diagram (FIG. 1, *D*). This figure shows merely the location of the nuclei and vacuoles. In very fine, slender hyphae nuclei may occur singly and widely separated. Here the cross walls are difficult to locate with certainty.

Preparations in which the conidia and fragments of sporogenous hyphae were stained by the same method indicate that there is also great variability in the number of their nuclei. Some of the larger cells at the base of branches made up of spore chains may contain four nuclei. These cells germinate the same as conidia under proper conditions. One frequently finds chains made up of *Oidium*-like cells, each cell containing a pair of nuclei (FIG. 1, *E*). Occasionally a conidium contains only a single nucleus. In this case the spore is rather small. One is inclined to assume that, since the nuclei occur so frequently in pairs in the homothallic structures, the pairing must be due to something which corresponds to conjugate division, particularly as about 75 per cent of single-conidium mycelia are bisexual. On the other hand, when one stains conidia developed on unisexual mycelia, he finds many binucleate spores. In fact, it is rather difficult to find spores with only one nucleus. This is especially true for conidia developed in potato dextrose cultures, where the hyphae, and therefore the conidia, are rather coarse or thick. It would be difficult to tell by the number of nuclei in the conidia whether the preparations were made from conidia formed on bisexual mycelia grown on cornmeal agar cultures or from conidia developed from unisexual mycelia grown on potato dextrose agar.

Since the binucleate conidia borne on unisexual mycelia must be unisexual, it cannot be denied that a binucleate condition may exist merely by the inclusion of two sister nuclei in each cell. This might be due to the fact that each cell originally contains a

single nucleus which later divides; which suggests perhaps that the theory of conjugate division in mycelial cells of the bisexual strains does not necessarily hold. To this it may be objected that if the nuclear divisions were merely simultaneous or occurred irregularly and the nuclei were distributed in the conidia merely by chance, one ought to find more than 25 per cent of the conidia unisexual. The evidence presented here certainly favors the idea that something corresponding to conjugate nuclear division in cells of the homothallic mycelia does take place. Mature unisexual ascospores of this species are binucleate. It would seem that full-sized conidia require at least two nuclei to maintain the nucleo-cytoplasmic equilibrium. A bisexual ascospore is much larger than are the unisexual ascospores, hence its four nuclei at maturity. It is very doubtful whether such a difference exists between unisexual and bisexual conidia.

Only a comparatively small number of preliminary culture experiments have been carried out in the attempt to answer the second question previously raised, namely, in mixed cultures containing two sexually reciprocal haplont mycelia of *Neurospora tetrasperma* are there anastomoses or fusions of unisexual hyphal cells followed by the production of secondary bisexual mycelia which produce homothallic or bisexual conidia? Using conidia obtained from fertile mixed cultures containing haplonts S<sub>0</sub> and S<sub>1</sub>, some 250 single conidium mycelia were isolated at various times. No evidence has been obtained so far to indicate that bisexual conidia are developed in such mixed cultures. Since haplont B produces a great many more conidia than haplont A, it was to be expected that a large proportion of the isolated mycelia would be sexually like haplont B. Only 3 out of the 250 were like haplont A. Since each of the unisexual mycelia would begin at once to produce its own kind of conidia in a mixed culture, it is very evident that even if later some bisexual conidia were developed they would be greatly outnumbered by the unisexual conidia produced when the culture was fresh. This would mean that results in order to be convincing must be obtained from a much larger number of cultures, which would not be at all necessary in proving that homothallic mycelia produce some unisexual conidia.

In conclusion it may be stated that the culture experiments reported here prove that a homothallic mycelium of *Neurospora tetrasperma* produces, in addition to the bisexual conidia to be expected, some unisexual conidia. No evidence has been obtained to show that bisexual conidia are produced in mixed cultures containing two heterothallic mycelia of opposite sexuality.

BUREAU OF PLANT INDUSTRY,  
WASHINGTON, D. C.

## NEW AND NOTEWORTHY FUNGI—V<sup>1</sup>

JOHN DEARNESS

### DEUTEROMYCETES

#### SPHAEROPSIDALES

##### *Stagonospora Amorphae* Dearn. & Bisby, sp. nov.

Pycnidia scattered, covered by the epidermis which is ruptured only by the slight ostium, rather flat, mostly elliptical, reaching  $340 \times 180 \mu$ , depth  $100 \mu$ ; wall dark, thin, membranous, thicker on top. Conidia almost sessile on the thin cellular layer lining the wall, hyaline, cylindric, 4-celled, usually 2 oil drops in each cell,  $42-56 \times 4-6 \mu$ .

Apparently causing "die-back" of green twigs of *Amorpha fruticosa*; Winnipeg, Man.; Aug. 1925; Sept. 1927. G. R. Bisby: 2547, 3633. (D. 5924.)

##### *Stagonospora lapidoviridis* sp. nov.

Spots subcircular or irregular, becoming confluent, slaty-green or watery-green, retaining their color after the leaf has become yellow or sere, .5-2 cm. Pycnidia innate, opening on the upper side, hyaline,  $30-120 \mu$ ; wall thin, obscure, a single layer of hyaline cells. Conidia few, large, lunate, hyaline, sometimes granular,  $22-36 \times 8-15 \mu$ , 3-celled, the middle cell always the largest, sometimes as much as  $20 \mu$  long; central conidiophores short, outer ones up to  $30 \times 3 \mu$ .

Parasitic on leaves of *Smilax herbacea*; Byron, Ont.; Sept. 25, 1924. Dearness: 5676.

*S. Smilacis* has ovate spores,  $11-22 \times 6-7 \mu$ .

##### *Stagonospora Phaseoli* Dearn. & Barth. sp. nov.

Spots arid or whitish with red border on upper side of the leaf, similar but duller below, scattered and becoming numerous, mostly circular, 5-10 mm. in diameter. Pycnidia black, innate, conspicuous above, can be seen from below, subglobose,  $150-225 \mu$ , often with a few bristles around the mouth; wall thin, of brown cells,  $7-9 \mu$ ; bristles when present 4-10, septate, obtuse,  $50-170$

<sup>1</sup> Continued from MYCOLOGIA 18: 255. 1926.

$\times 4 \mu$  at top and  $8-10 \mu$  at base. Conidia hyaline, subcylindric, continuous to 5-septate, grumous and guttulate,  $21-37 \times 4-6 \mu$ .

Parasitic on cultivated beans—*Phaseolus vulgaris*—and associated on most of the leaves with *Cercospora canescens*; Greenfield, Tenn.; Sept. 28, 1925. E. Bartholomew: 9290. (D. 5755.)

**Hendersonula pinicola** sp. nov.

Stromata dark brown, covering the full width of the needle and when confluent resembling the dark crust of *Hypoderma* (*Bifusella*) *lineare*. Loculi scattered,  $110-135 \mu$  in diameter, visible as pustules rising above the level of the crust. Conidia dark-amber color, fusoid, mostly 2-septate, slightly constricted, sessile,  $12-15 \times 3-3\frac{1}{2} \mu$ .

On partly green needles of *Pinus Strobus*; Davidson River, N. C.; Aug. 6, 1925. G. G. Hedgcock: 43031. Also at Brevard, N. C., and Roan Mtn., Tenn. "A needle blight cast"—G. G. H. As this was mixed with other species its parasitism cannot be strictly defined. (D. 5864.)

**Camarosporium Betulae** Dearn. & Barth. sp. nov.

Pycnidia scattered, developing in the phloem and raising the epidermis into truncate-conical pustules, .6-.8 mm. in diameter at the base, opening by a large perforation,  $200 \mu$ , through a white or pale-buff disk; sometimes in small, confluent, *Dichomera*-like groups. Conidia brown, ovate to oblong-elliptic, 3-5-septate, longitudinal septa joining one or two pairs of transverse ones, rarely extending more than half the length, many merely phragmosporous,  $15-24 \times 5-9 \mu$ , sessile on a rather thick, white, cellular lining of the wall.

On dead branchlets of *Betula populifolia*; Queenston, Ont.; July 21, 1913. E. Bartholomew: 5102. (D. 4579.)

**Septoria Boycei** sp. nov.

Spots pale reddish-brown above, duller below, scattered, numerous, circular at first, 1 mm. in diameter, extending between the large veins, seldom crossing them, reaching 7.5 mm. Pycnidia epiphyllous, thinly scattered, dark, small,  $40-90 \mu$ , many of them rather widely open at the top; wall thin, of one layer of quadrate cells. Conidia hyaline, strongly curved or flexuous,  $30-70 \mu$ , mostly between  $45$  and  $60 \mu$  by  $2$  to  $2\frac{1}{2} \mu$ , 1-3-septate.

Parasitic on leaves of *Betula fontinalis* Sarg.; Clackamas Co.,



Oreg.; alt. 4,000 ft.; July 8, 1924. J. S. Boyce: 1291. (D. 5670.)

This is different from each of the four *Septoriae* on *Betula* described in the Sylloge. The pycnidia are so widely open in some of the spots that they might be sought in *Phleospora*.

**Septoria Ceanothi** sp. nov.

Spots scattered, circular, numerous, small, 1 mm., whitish, red-bordered, similar on both sides. Pycnidia black, punctate, one or a few on a spot, mostly single and central, epiphyllous, 100–120  $\mu$ . Sporules hyaline, curved, 20–36  $\times$  1  $\mu$ .

Parasitic on leaves of *Ceanothus sanguineus* Ph.; Bonner Co., alt. 3,000 ft.; July 27, 1924. J. S. Boyce: 1289. (D. 5669.)

*Cylindrosporium Ceanothi* Ellis & Ev. On dark spots has spores 4  $\mu$  wide.

**SEPTORIA LYCHNIDIS** Desm.

On living leaves of *Lychnis alba*; Mattituck, N. Y.; Oct. 1, 1923. Roy Latham. (D. 5442.)

Spots brown-red. Conidia 40–65  $\times$  2½  $\mu$ , 5–7-septate. This European species does not seem to have been hitherto reported in America.

**Septoria pinicola** sp. nov.

Pycnidia amphigenous, mostly on the external side of the needles, scattered, often serried in short lines, the black top barely visible through narrow cleft of the slightly pustuled epidermis, seated in the browned or blackened hypoderm, globose, 90–165  $\mu$ ; wall black, 35–55  $\mu$  thick. Conidia hyaline, granular and often with a row of minute guttae, straight or curved, one or both ends somewhat narrowed, continuous or uniseptate, 30–60  $\times$  3  $\mu$ , mostly between 45 and 50  $\mu$  long, on short conidiophores arising from a thick, rather compact pseudo-parenchyma lining the wall.

On blighted needles of the lower limbs of forest trees, *Pinus virginiana*; Chain Bridge, Va.; May 10, 1927. G. G. Hedgcock: 3455. (D. 5837.) There were no green or partly green needles on the twigs received. While the general appearance of the needles was brown, those bearing numerous pycnidia were grayish and often dark-banded. If *Septoria spadicea* Patterson & Charles is a very variable species, this may be an extreme form of it.

**Septoria septoriopsidis** Dearn. & Overh. sp. nov.

Spots pale reddish-brown, immarginate, circular or irregular, 4–10 mm., surrounded as seen when held to the light with a yellowish or discolored area 2 to 4 mm. wide, similar on both sides. Pycnidia strictly epiphyllous, in the palisade tissue, 60–115  $\mu$ , globose at first, becoming saucer-like and superficial and then bearing dense, white tufts of conidia simulating *Septoriopsis* (*Cercoseptoria* Petrak), Mycologia 11: 4. Conidia straight or slightly curved, 1–3-septate,  $14\text{--}27 \times .5\text{--}75 \mu$ , on a layer of cells and filiform sporophores about 30  $\mu$  thick.

Parasitic on leaves of *Betula lenta*; Center Co., Pa.; July 28, 1924. R. S. Kirby and L. O. Overholts: 9547. (D. 5705.)

**Septoria Shepherdiae** (Sacc.) Dearn. comb. nov.

*Cylindrosporium Shepherdiae* Sacc. Ann. Myc. 11: 551. 1913.

Dr. Saccardo founded this species on a Field, B. C. collection of leaves of *Lepargyrea canadensis* which I sent him in 1912. (D. 3477.) In an Idaho collection of the same species, J. S. Boyce: 1290, as well as in stained trans-sections of the co-type, I find a thin undarkened pycnidial wall. The species should be classified as a *Septoria* and the width of the conidia stated as 2  $\mu$  instead of 3–4  $\mu$  unless the jelly-sheathing be included.

**Septoria Sonchi-arvensis** Dearn. & Bisby, sp. nov.

Spots round, 2–4 mm., with broad, red-brown margin. Pycnidia mostly epiphyllous but variable in this particular, dark, few on a spot, 150  $\mu$ . Conidia hyaline, septate,  $20\text{--}42 \times 2\frac{1}{2} \mu$  at one end and  $1\frac{1}{2} \mu$  at the other, sometimes even.

On *Sonchus arvensis*; Winnipeg, Man.; June–Sept. 1925. Bisby and Conners: 1573, 2379. (D. 5868.) *S. sonchifolia* Cooke is also existent in the same region.

**Septoria sitchensis** sp. nov.

Spots small, 2–4 mm. across, limited by the veinlets, not bordered, widely scattered, red above, yellow below and blackened by the pycnidia if they happen to be close together. Pycnidia usually few on a spot, large, reaching a width of 225  $\mu$  and a depth of 150  $\mu$ , innate, visibly bulging the cuticle on the lower side of the leaf, reaching and opening through but not elevating the cuticle on the upper side; wall 33  $\mu$  thick at base; under the hand lens seeming to be hypophyllous. Conidia hyaline, curved or flexuous,  $45\text{--}90 \times 3 \mu$ , attenuating towards the distal end to

1.5  $\mu$ . The conidia continuing adnate to their conidiophores with the basal layer can be removed from some of the pycnidia and transferred in liquid from place to place.

On living leaves of *Pyrus sitchensis* (Roem.) Piper; Upper Priest River, Ida.; July 30, 1924. C. R. Stillinger: 2142. Beaton, B. C.; Aug. 25, 1926. J. S. Boyce: 1670. (D. 5802.)

**Rhabdospora aristata** Dearn. & Barth. sp. nov.

Pycnidia scattered, sub-cuticular, dark brown, firm, ostiolate, hemispheric to conic, sometimes centrally depressed, .25-.5 mm. Sporules hyaline, consisting of a narrow-fusoid, 1- to 4-septate, weakly curved portion,  $20-30 \times 2\frac{1}{2}-3 \mu$ , basally contracting into an acuminate, curved, aristiform pedicel,  $20-30 \times .5 \mu$ .

On dead stems of *Heracleum lanatum*; Choteau, Mont.; Aug. 9, 1917. J. A. Hughes, J. R. Weir: 9183. (D. 4591.)

**Rhabdospora Eucalypti** Dearn. & Barth. sp. nov.

Pycnidia thickly scattered, erumpent, 100-150  $\mu$ , contents gray in section; wall well developed above, thinning out below; ostiola black, papillate. Conidia hyaline, linear, curved or hamate,  $19-22 \times .75-1 \mu$ , on linear conidiophores nearly as long and mixed with tongue-like extensions.

On dead stems of *Eucalyptus* sp.; San Francisco, Cal.; Aug. 2, 1924. E. Bartholomew: 8859. (D. 5726.)

#### LEPTOSTROMATALES

**Leptothyrium Pseudotsugae** sp. nov.

Sporocarps black, thickly scattered, circular, perforate, not discoloring the leaf, 60-100  $\mu$ , many of them infertile. Conidia hyaline, globose, oval or elliptic,  $6-8 \times 4-6 \mu$ .

A "fly-speck" fungus on living leaves of *Pseudotsuga taxifolia*; Stonewall Gap, Colo.; June 18, 1917. Hedgcock and Bethel: 24,396. (D. 5698.)

*Rhabdogloeum Pseudotsugae* Syd. on the same collection.

**Leptothyrium stenosporum** sp. nov.

Pycnidia black, scattered or grouped, circular, often centrally perforate, sub-hemispheric, mostly on paler portions of the inner faces of the needles,  $\frac{1}{4}-\frac{1}{2}$  mm. Conidia hyaline, minute, allantoid,  $2\frac{1}{2}-4 \times .75-1 \mu$ , on fascicled or branched conidiophores up to 9  $\mu$  long, branches .5  $\mu$  thick.

On blighted needles of small trees, *Pinus Strobus*; Elijay, Ga.; July 27, 1925. G. G. Hedgcock: 11,998. (D. 5904.)

**Leptostroma Hedgcockii** sp. nov.

Sporocarps same color, shape and size as the apothecia of *Hypoderma hedgcockii* Dearn. Conidia snow-shoe shaped, hyaline, with one large nucleus or two smaller ones in the wider (upper) end,  $16-24\ \mu$  long by  $4-6\frac{1}{2}\ \mu$  wide in the upper half, contracting to  $2-3\ \mu$  at the lower end; on branched or fascicled conidiophores in units up to  $30\ \mu$  long, the branches  $2\ \mu$  thick.

On living needles of *Pinus rigida* Mill; Andrews, N. C.; July 19, 1925. G. G. Hedgcock: 11,952. (D. 5827.) Collected in as many localities as the ascigerous state; apparently more common. (Mycologia 18: 240.)

(?) **Leptothyrella Laricis** sp. nov.

Leptostromes elongated, narrow,  $.25-1 \times .1\ \text{mm.}$ , widely cleft, on the inner side of the needles. Conidia hyaline, oblong, usually short-pointed at the ends, 2-septate, guttate,  $12-24 \times 4-5\ \mu$ , mostly  $16-17 \times 4\frac{1}{2}\ \mu$ .

Parasitic on needles of *Larix occidentalis* Nutt.; Moscow, Idaho; Sept. 23, 1911. G. G. Hedgcock: 9541. Also 11,117 and other collections. (D. 5691.)

This species in its form is intermediate between *Leptothyrella* and *Cystothyrium*. It is pretty certainly the conidial stage of *Hypodermella Laricis* Tub. var. *octospora* with which it is associated on some of the needles.

**Leptostromella Cassiae** Dearn. sp. nov.

Pycnidia black, numerous, very thickly scattered, covered by the thin, translucent cuticle, minute, circular to oblong,  $.2-.5\ \text{mm.}$  Spores nearly straight to hamate-curved, hyaline,  $18-25 \times 1-1\frac{1}{2}\ \mu$ .

On dead stems of *Cassia marilandica* L.; Fort Ann, Washington Co., N. Y.; June 1915. S. H. Burnham: 36. (D. 4040.)

**Dinemasporium corrugatum** Dearn. & Barth. sp. nov.

Pycnidia black, open, setose at base,  $80-150\ \mu$ , corrugate, involute. Conidia hyaline, widely lunate, ciliate at each end,  $5-8 \times 2.75\ \mu$ , cilia  $3-6 \times \frac{1}{2}\ \mu$ ; conidiophores fasciculate.

On decorticated *Morus alba*; Stockton, Kan.; March 1924. E. Bartholomew: 8653. (D. 5544.)

## MELANCONIALES

**Gloeosporium melleum** Dearn. & Overh. sp. nov.

Spots amphigenous, circular, 2–5 mm., becoming confluent, cream-colored to dull yellow, mostly obscurely concentrically ridged, the slightly raised border concolorous or somewhat darker. Acervuli amphigenous, or on some spots epiphyllous only, the smaller ones dark and *Phyllosticta*-like, the larger, especially on the lower side of the leaf, honey-colored, 50–210  $\mu$ , usually segregated near the middle of the spot. Spores exceedingly numerous, minute,  $2\frac{1}{2}$ –3  $\times$  .5  $\mu$ .

On living leaves of *Chenopodium album*; Pottstown, Pa.; July 7, 1924. R. S. Kirby and L. O. Overholts: 9690. (D. 5746.)

*Gl. Chenopodii* has spores 8–9  $\times$  3–4  $\mu$  *fide* Saccardo, Syll. Fungi 10: 44.

**Gloeosporium multipunctatum** sp. nov.

Spots extensive, immarginate, mostly centered along strong veins for 1–3 cm., brownish, darker above, particularly while the leaf remains green. Acervuli mostly hypophyllous, position visible on upper side, innate, very numerous, more so near the veins, slightly rising above the leaf-level and appearing like minute beads of glue, 20–200  $\mu$ . Conidia hyaline, oblong, straight, 6–9  $\times$   $1\frac{1}{4}$ – $1\frac{1}{2}$   $\mu$ .

On green and languishing leaves of *Acer saccharinum* L.; Pond Mills, Ont.; Oct. 7, 1924. Dearness: 5678.

**Rhabdogloeum abietinum** sp. nov.

Affected leaves scattered among green ones, yellowed or sered throughout. Acervuli mostly hypophyllous, raising the epiderm into circular or elongate, concolorous blisters, .5–2 mm. Conidia hyaline, continuous, fusoid, often somewhat curved, 15–21  $\times$  4–5  $\mu$ , on dendriform conidiophores, 1  $\mu$  thick, in units about 45  $\mu$  high.

Parasitic on needles of *Abies fraseri* (Pursh) Lindl.; Mt. Mitchell, N. C.; Aug. 10, 1925. C. F. Kerstian, G. G. H.: 43,026. (D. 5902.)

**Myxosporium megallanto** sp. nov.

Acervuli scattered or in series, pustular, sub-epidermal, circular, .5–.7 mm., extending or becoming confluent in sub-parallel lines and raising the epidermis into narrow, low, pale-gray ridges, .5–3 or 4 cm. long by .2–.5 mm. wide at the base.

Conidia hyaline, granular, large sausage or cucumber shape, some straight but oftener curved usually more strongly above,  $45-50 \times 12-16 \mu$ , either on short conidiophores,  $5-15 \times 5-6 \mu$ , or terminal on a chain of cells each  $5-20 \times 5-8 \mu$ , total length  $15-100 \mu$ , widening upwards, quite probably a chain of incipient conidia. Overtopping the conidia are linear, septate, hyaline paraphyses,  $40-150 \mu$  long,  $3 \mu$  wide at the top and  $6 \mu$  at the base.

On dead branchlets of *Liriodendron tulipifera*; Southold, N. Y.; Apr. 1923. Roy Latham: 1803. (D. 5526.)

This is an inconspicuous fungus but when opened up is as attractive as it is peculiar. It is anomalous in *Myxosporium*. The total central depth of a large acervulus reaches  $240 \mu$ .

***Myxosporium negundicolum* Dearn. & Barth. sp. nov.**

Acervuli thickly scattered, on some of the twigs so thickly as to blacken them by the spore masses showing through the translucent cuticle, .4-.8 mm. Conidia hyaline, densely granular, some of them also guttate, sometimes shortly catenate,  $24-28 \times 9-12 \mu$ , on rather stout conidiophores.

On dead shoots of *Acer negundo*; Blue Rapids, Kan.; June 25, 1925. E. Bartholomew: 9112. (D. 5874.)

***Myxosporium roseum* Dearn. & Barth. sp. nov.**

Acervuli thinly scattered, seated in the cortex, raising the epidermis into round or sub-elongate, centrally depressed pustules, .5-2 mm., from which often issue salmon-rosy cirrhi. Conidia hyaline, oblong, with rounded ends,  $15-18 \times 5 \mu$ , on simple or branched conidiophores  $15-18 \times 2 \mu$ .

On dead branchlets of *Ulmus americana*; Stockton, Kan.; Nov. 1923. E. Bartholomew: 8602. (D. 5399.)

***Colletotrichum Viciae* Dearn. & Overh. sp. nov.**

Part or all of the leaf yellowed or whitened. Acervuli scattered, amphigenous, more numerous on the upper surface, as many as 20 on a sq. mm., melleous,  $50-90 \mu$  in diameter, but also large and irregular by confluence. Setae lacking or few or numerous, hyaline to fuliginous, reaching a length of  $65 \mu$ ,  $6 \mu$  at base, tapering to an acute tip, continuous or 1-septate near the base. Conidia hyaline, narrowly crescentic, nucleolate,  $17-21 \times 3-4 \mu$ .

Parasitic on *Vicia* (?) *villosa*. State College, Pa.; July 14, 1924. C. R. Orton: 9335. (D. 5627.) Affinitive to *C. carpophilum* Kell. & Swingle.

**Marssonia Sonchi** Dearn. & Bisby, sp. nov.

Spots 3–5 mm., amphigenous, cinereous-brown, purplish-red-bordered, obscurely concentrically zoned, surrounded by a diffuse discoloration 2–4 mm. wide. Acervuli 80–300  $\mu$ , mostly epiphyllous, circular or irregular, nearly concolorous, slightly convexing the cuticle. Conidia escaping by a minute perforation, hyaline, 1-septate, constricted, usually one or two guttae in each cell, oblong-elliptic, 10–13  $\times$  3–5  $\mu$ .

On living leaves of *Sonchus arvensis* L. Common in locality of collection. Manitoba Agric. College, Winnipeg; June–Aug. 1924. G. R. Bisby: 1816, 2038. (D. 5793.)

**Septogloeum rhopaloideum** Dearn. & Bisby, sp. nov.

Spots scattered, numerous, small and limited by the veinlets at first but soon becoming confluent and spreading to occupy most or all of the leaf, watered-gray, more cinereous above. Acervuli hypophyllous, large, numerous, 10–20 per sq. mm., convex until the cuticle ruptures, then dingy-melleous, 200–300  $\mu$ . Conidia often emerging in tongue-like masses, hyaline, Indian-club or tadpole shape, pointed end outward, 2–4-celled, mostly 3-celled, 40–60  $\times$  8–12  $\mu$ , the proximal cell short, 6–9  $\times$  5–7  $\mu$ , middle cell largest, 21–30  $\times$  8–12  $\mu$ , distal or tail cell 15–24  $\mu$  long and acuminate to a point.

Parasitic on leaves of *Populus tremuloides*; Winnipeg, Man.; Aug. 1924. G. R. Bisby: 1818, 2127. (D. 5794.)

**CORYNEUM MICROSTICTUM** Berk. & Br. var. *foliae* Dearn. & Overh. var. nov.

A *Coryneum* on cultivated rose leaves with brown, 3-septate spores, 12–15  $\times$  5  $\mu$ , is taken, on morphological grounds only, to be a variety of *C. microstictum* B. & Br., a species producing an injurious canker on rose stems.

The leaf tissue is killed in and beyond the brown-bordered spots. The black, irregular acervuli, 70–200  $\mu$ , rupture the cuticle on the upper side. Wrinkling of the cuticle near the margin of the spot causes one or more broad, whitish lines concentric with the raised border.

Parasitic on foliage of *Rosa* sp., cult.; Newcombe, N. Y.; Aug. 8, 1924. C. R. Orton and L. O. O. Overholts: 9714. (D. 5608.)

CORYNEUM SEPTOSPORIOIDES (Ellis & Ev.) Sacc. & Syd.

*Coryneum Negundinis* Ellis & Ev. Bull. Torrey Club 24: 292. 1897.

On *Acer negundo*. Collections on still living branches by E. Bartholomew, Ten Sleep Canyon, Wyo.; H. F. Perkins, Prince Albert, Sask.; W. P. Fraser, Saskatoon, show that this fungus can produce a serious canker on branches of the ash-leaved maple. The original description locates it on dead limbs. These had probably been killed by it.

The hyphal features vary in sections from different regions of the canker; some of them suggest *Septosporium*—erect sterile hyphae are to be found—and others *Clasterisporium*. (D. 5430.)

**Monochaeta pinicola** sp. nov.

Acervuli scattered, amphigenous, black, sub-epidermal, erumpent, punctate, .1 mm., to narrow elongate, 1 mm., sometimes confluent. Conidia ciliate-pedicellate and similarly ciliate at the upper end, curved, brown, 4-celled, the two middle cells dark brown, the other two pale brown and truncate-conic, width at middle septum  $5\frac{1}{2}$ – $8\frac{1}{2}$   $\mu$ , at the attachment of the cilia 3  $\mu$ , length exclusive of the cilia 14–19  $\mu$ , the cilia 7–14  $\times$  .75  $\mu$ .

On blighted needles of *Pinus palustris*; Hogan, Fla.; March 1918. G. G. Hedgcock: 25,156; on *P. echinata*. G. G. H.: 24,395. (D. 5863.)

**Cryptosporium acicolum** Thüm.

*Septoria acicola* (Thüm.) Sacc. Syll. Fung. 3: 507. 1884.

Parasitic on needles of *Pinus palustris*, Brooksville, Fla., Mar. 11, 1915—(G. G. H.: 17,424)—and in more than twenty other collections made by G. G. Hedgcock on *Pinus* spp.—*taeda*, *echinata*, *glabra*, *virginiana*—in five of the southern States. Labelled "Red spot; common and injurious on young forest trees." (D. 5830.)

There can be found in these collections exemplification of the features of the above-named fungus as described in the Syll. Fungi 3: 507.

If this is the fungus de Thümen had in hand, the description in the Sylloge may be supplemented as follows:

Acervuli amphigenous, globose at first, 50–100  $\mu$  in diam., becoming elongate up to 1.5 mm., seated in the mesophyll,



discharging spores through a cleft of the epidermis. Basal layer of colored, compact pseudoparenchyma. Conidia simple and sub-hyaline, becoming 1-3-septate and brownish,  $19-32 \times 3\frac{1}{2}-4\frac{1}{2} \mu$ , usually curved more in one half than in the other. The acervuli are found in the red spots and often in the browned portion beyond the spot.

While not a typical *Cryptosporium* this fungus fits better there than in *Septoria*. Its intimate association with the "red spot" is ground for suspecting causal relation. If it be the cause of the spotting and browning of the pine foliage, it is an important economic fungus. *Oligostroma acicola* Dearn., Mycologia 18: 252, may be an ascigerous relative.

***Cylindrosporium sibiricum* Dearn. & Bisby, sp. nov.**

Spots amphigenous, similar, red-bordered in the green leaves, blue-gray in the yellowed ones, circular, small, 1-3 mm., becoming irregular. Acervuli numerous and minute as if issuing at almost every leaf-stoma, later marked by fewer, large amphigenous cirrhi. Conidia hyaline, 1-3-septate, straight or somewhat curved,  $22-45 \times 2.75-3.25 \mu$ , mostly about  $30 \times 3 \mu$ .

On living leaves of *Apocynum sibiricum* Jacq.; Pierson, Man.; June 30, 1921. V. W. Jackson. (D. 5661.)

***Cryptosporium Boycei* sp. nov.**

Acervuli scattered, seated in the cortex, not reaching or marking the wood, nearly circular, .5-1.5 mm. in diameter, .5-.8 mm. high, encinctured by the upturned epidermis and rising at most  $280 \mu$  above the surrounding bark; surface pulverulent-yellow-gray when fresh, becoming dull gray-brown, the basal layer either simply lining the cavity or by invaginations appearing locellate. Conidiophores  $20-90 \times 2.5 \mu$ , usually curved at the tip after the conidia are discharged, and like the layer from which they arise yellow in the mass. Conidia hyaline, yellow in the mass, acuminate-acute at each end, crescentic, curved to a semicircle or strongly falcate, sometimes incurved at the outer end, 3-septate,  $45-75 \times 3.5-5 \mu$ .

Producing a canker on languishing branches and small trunks of *Pseudotsuga taxifolia*; Stanley Park, Vancouver, B. C.; Aug. 24, 1924. North Bend, King Co., Wash.; July 31, 1927. J. S. Boyce: 1285, 1766. In Herb. U. S. For. Path. 40,394. (D. 5666.)

Dr. Boyce studied this fungus in King Co. on a stand of Douglas

fir reproduction averaging about 10 feet in height. He found that trees showing winter injury—parch blight—were generally more or less cankered, in some instances from the top nearly to the root, and that on the severely injured and dead bark the fungus was fruiting abundantly. He concluded that it attacks only the injured or weakened trees and that there was no evidence of perennial progression into the healthy tissues.

***Libertella ulmicola* Dearn. & Barth. sp. nov.**

Acervuli large, irregular, up to 1 cm. long, sometimes nearly as wide and sub-circular, in the liber of elm posts. Conidia hyaline,  $15-24 \times 1 \mu$ , issuing in large yellow or orange wedges, up to 1 cm. by 4 mm.

In the bark of *Ulmus americana*; Stockton, Kan.; March 1925. E. Bartholomew: 9023. (D. 5882.) *L. Ulmi-suberosae* Oudem. has different spores.

LONDON, ONTARIO,  
CANADA

## NOTES AND BRIEF ARTICLES

Professor A. H. Chivers, head of the Department of Botany in Dartmouth College, has recently spent a semester on sabbatic leave in the Department of Plant Pathology in Cornell University.

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Guy West Wilson, for several years Professor of Biology at Upper Iowa University, Fayette, Iowa, has been appointed and served during the year as Professor of Botany at Penn College, Oskaloosa, Iowa.

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Dr. B. O. Dodge, who has been for the past eight years pathologist of the Bureau of Plant Industry of the United States Department of Agriculture, has accepted an appointment as plant pathologist of The New York Botanical Garden and began work at the Garden on May 1st. Dr. Dodge was assistant and instructor in the Department of Botany of Columbia University from 1909 to 1920.

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In "The Rusts and Smuts of Bermuda" by Whetzel and Jackson the statement is made regarding *Entyloma Meliloti* McAlpine on *Melilotus indica*: "The species appears to be common on this host everywhere in the islands during the spring months; known otherwise only from Australia." *Trans. British Mycological Society* 13: 6 (1928). The parasite was found on this host at Auburn, Alabama, in March 1921 by J. F. Duggan.  
J. J. DAVIS

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Manuscript is coming in so much more rapidly than it can be published in MYCOLOGIA that contributors are requested to cut down their articles to the briefest possible form. They are also requested to use greater care in citations to literature, making

them brief and consistent and, thereby, economizing the time of the Editor and increasing the likelihood of an early publication of the contribution.

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Following the publication of my article in the March-April issue of MYCOLOGIA, Mr. E. W. Mason of the Imperial Bureau of Mycology, Kew, England, calls attention to the following facts pertaining to *Sphaerostilbe longiascus* Möller which is there regarded as a synonym of *Macbridella striispora*: The genus *Calostilbe* was established by Saccardo and Sydow (Sylloge Fung. 16: 591. 1902) for Möller's species, which was made *Calostilbe longiasca* (Möller) Sacc. & Syd. Since this species is synonymous with our species, *Macbridella striispora* becomes *Calostilbe striispora* (Ellis & Ev.) comb. nov. This genus takes from *Macbridella* the forms with the stilbaceous conidial stage. FRED J. SEAVER.

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### The North American Cup-fungi

(Operculates)

#### ADVANCE NOTICE

The above work, which has been in the course of preparation for a number of years, is well under way and is expected to appear within the year, unless delayed by some unforeseen difficulty. It is estimated that the book will comprise 250 pages or more of text, forty-six plates and a number of text figures, and will sell for five dollars. There is no other monograph of this group of fungi in America and, while this is not being offered as the "last word" on the subject, it is, at least, the beginning of a more complete knowledge of these interesting forms of plant life. That there is need of such a work is indicated by the fact that one hundred advance orders were placed within thirty days after the project was announced, and that without any advertising other than a few circular letters sent out each morning in the course of the day's routine. As the size of the edition will be regulated somewhat by the rapidity with which advance orders are received, institutions and individuals wishing copies should place their requests at once. FRED J. SEAVER.

### Gäumann's Comparative Morphology of Fungi

American students of mycology will welcome the appearance of the English translation of Professor Albert Gäumann's "Comparative Morphology of Fungi" prepared by Dr. Carroll W. Dodge of Harvard University. The German edition of this work appeared in 1925 and has been much used by advanced students in the larger universities of this country. The English edition by Dr. Dodge will make it much more accessible and highly prized as a text.

While it is a translation, as stated by the translator, it is a loose translation of the German, and an attempt has been made to correct some of the misconceptions of the original author, as well as to incorporate much information of a morphological nature which has come to light since the German edition appeared, so that it is really much more than a translation.

The book, which is put out by the McGraw-Hill Book Company of New York City, consists of 701 pages of text and 406 text figures. A copy of this work should be in every mycological library. FRED J. SEAVER.

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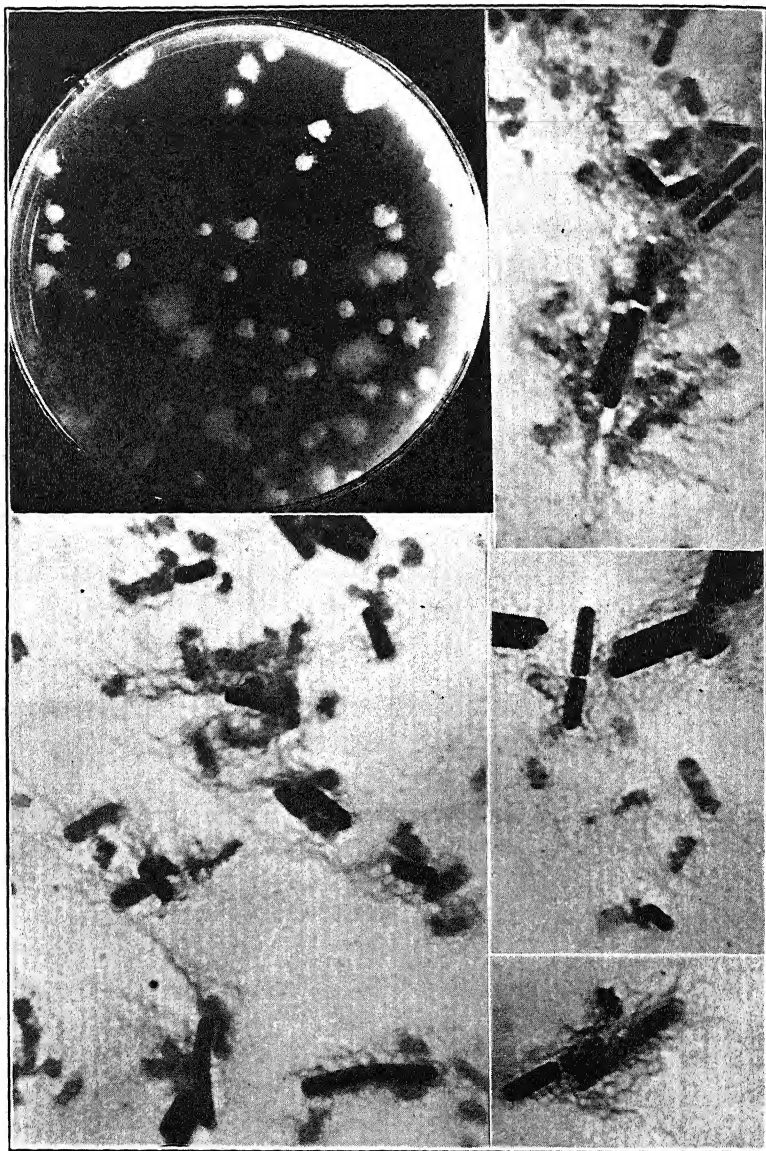
### Book Review

Late in 1927 there came from the press a book that will prove interesting to all American students of the fleshy fungi. It is entitled "Mushrooms and Toadstools" and is written by Messrs. H. T. Güssow and W. S. Odell of the Dominion Experimental Farms, Ottawa, Canada. The preface states that the book "is not intended as a 'learned treatise,' but is meant to appeal to students as well as nature lovers." The book comprises a total of 274 pages, with special chapters on the general structure and development of fleshy fungi, the use of them as food, poisoning by mushrooms, and a brief account of mushroom culture. Rather complete descriptions are given of about 160 species of fleshy fungi commonly found in Canada. While this list is small in proportion to the number of species that must occur in a region of the extent covered by the book, yet for the amateur collector and the mycophagist it will be found to fill a definite need. The illustrations presented are ample, consisting in all of 128 half-tone

full-page plates that must be conceded to be the best half tones produced in any similar work in this country. Not the least attractive feature of the book is the price. It may be obtained from or through either of the authors for the price of one dollar.

That it cannot be used as a complete manual, and was not intended to be so used, is indicated by the fact that but seven species of *Clavaria* are included, four of *Hydnum*, and eight of *Boletus*. Yet even the advanced mycologist will find the illustrations of value, and the book is attractively bound and will grace any bookshelf. Errors due to misdeterminations seem to have been entirely eliminated—at least none have been noticed by the reviewer. In general the book is a distinct credit to its authors and they are to be congratulated in presenting us with a volume of this type. L. O. OVERHOLTS.





VARIATION IN BACTERIA



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## VARIATIONS WITHIN A BACTERIAL SPECIES—I MORPHOLOGIC VARIATIONS<sup>1</sup>

H. R. ROSEN

(WITH PLATES 29-33)

It would seem redundant to call attention to the fact that bacteria, including phytopathogens, may be expected to vary. Many articles, including extensive monographs, have been written on the subject; a symposium was devoted to it at the recent International Botanical Congress held at Ithaca, New York, and yet, in spite of this, the current literature on bacterial plant pathogens shows clearly that very little recognition has been given to this phenomenon. It is true that most of the work dealing with bacterial variability has appeared in medical journals and in other periodicals and books which are ordinarily not available to plant pathologists and mycologists; likewise, from a reading of the literature it is quite obvious that medical bacteriologists have paid very little attention to the literature of mycologists and plant workers in general and have not paid sufficient attention to morphological details. The article here presented and one to be given later will attempt in a limited fashion to bridge this gap and will call attention to several cases of variability within a species as it has been observed by the writer in certain bacterial plant parasites, in a human parasite and in several saprophytes.

What is meant by variation in a bacterial species? Is it something, more or less ephemeral, that is associated with or brought about by differences in environment, is it inheritable, or is it simply a phase or a part of a "life cycle"? Such questions are

<sup>1</sup> Research paper No. 83, Journal Series, University of Arkansas.

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not as likely to be asked by a biologist dealing with a variation in a pure line of tomatoes, for example, as they are by a bacteriologist. Why? Because in the first place it is a relatively simple matter to obtain a homozygous individual belonging to certain groups of higher plants or animals, compared with the difficulties of isolating a minute, single-celled individual, and, unless one does isolate a single bacterium and observes its offspring, how can it be certain that a pure line and not a mixed one has been obtained? In the second place, a number of bacteriologists now insist that the so-called fission-fungi have a far more complex life history than has been attributed to them in the past. Hence, it is not at all clear that any deviation may not represent a phase which, while differing from that obtainable under "normal" conditions, nevertheless represents a distinct part of the life history. To illustrate the point, it is very well known that, in various species of fungi, cultural conditions which make for so-called normal growth preclude the possibility of producing such structures as perithecia, zygospores, chlamydospores, sclerotia and other well-defined types of bodies. It is only when nutrients run low, or when the temperature is "abnormal" or when a chemical or physical stimulant is applied that certain structures are obtained which are really just as normal to the species at hand as the so-called normal phases. Would it not be more correct to say that when we isolate a microörganism and give it facilities for making profuse growth of a certain recognized type it is under abnormal conditions? How often under natural conditions will a given microörganism be found to have a substratum all to itself, or to have the same light for any length of time, the same temperature, or the same humidity? Even a pathogen, once it penetrates and occupies a portion of a host, must soon compete with secondary invaders for its supremacy in the occupied area, and may not the secondary invader so check the primary one as to inhibit its further invasion into unoccupied territory? The writer has evidence to show that this happens at times to *Bacillus amylovorus* and that a yellow schizomycete, entirely unrelated to the fire blight pathogen, is with astounding frequency to be found following up the work of the primary parasite and apparently limiting it in the amount of host tissue

invaded. This has been found to occur not only in young, succulent pear and apple twigs, but also in green pear fruit. In passing it may be worth while to note that this is a field of endeavor that has hardly been touched in spite of the fact that it gives promise of yielding extremely interesting scientific as well as practical knowledge. We are so anxious to obtain the parasite and nothing but the parasite in pure culture that we have not taken the trouble to find out something about the natural companions of the pathogen and whether they are desirable or undesirable from the standpoint of the parasite and from the standpoint of the host. In brief, when we speak of normal growth or of normal growing conditions of a bacterium or a fungus we may be designating a more or less abnormal and unnatural state, and, to cite a specific case, when it is found that perithecia of *Venturia inaequalis* are not produced in pure cultures unless the mycelium is violently treated, either severely cut into bits or subjected to the action of a contaminant, we do not assume that these structures are abnormal or atypical. Likewise, it is highly improper to speak of abnormal or involution forms in bacteria because they are produced under conditions which are not considered ideal for the production of a standard type of growth. But, while a great deal has been written about various types of structures in bacteria, such as gonidia, buds, sex organs, zygosporos, symplasm and the like, they are still shrouded in mystery in spite of the splendid work of a relatively large number of bacteriologists, including Löhnis (28, 29, 30), Hadley (22), Pringsheim (39), Enderlein (17), Mellon (32, 33, 34), Gurney-Dixon (21), Hort (23, 24, 25), Baerthlein (3), and Eisenberg (10 to 16). In view of this uncertainty bacteriologists cannot easily decide whether a given form, designated a variant, is comparable to those variants in pure line work in higher plants and animals or whether it is merely another stage in the life history of the organism.

Assuming that bacteria have a far more complex life history than has formerly been recognized and that they are pleomorphic or "cyclogenic," is there not still the possibility that, given any one form or phase of the cycle we may expect it to show variation? In other words, granted that one phase or form of a particular species may produce gas and another phase of the

same species show no gas production, the possibility still exists that there may be clones or strains of the organism which in comparable phases may or may not produce gas or which may vary in a number of other particulars, morphologically and physiologically. Hadley (22), who has written a splendid monograph on variation in bacteria, prefers not to think of it from this viewpoint. "Not until we have become able to recognize the wide range of cyclogenic variation to which all bacterial species are susceptible, shall we be able to detect the permanent departure from the specific cycle" (p. 273). Then again, "The most important point . . . is that the cyclogeny of a single bacterial species embraces many strictly normal forms of culture growth, each of which is endowed with different biochemical, serologic and antigenic characters" (p. 272).

The possibility that within a recognized species of bacteria there may be a number of races, strains or varieties has been set forth from time to time by various bacteriologists. Some twelve years ago Cole and Wright (6) discussed this subject, applying the pure line concepts of geneticists to bacterial species. They present an excellent summary of some of the literature on this subject, paying special attention to the application of the statistical method by Andrews and Horder, Bredemann, Goodman, Winslow, Winslow and Walker, Wolf, and Buchanan and Truax, in the study of bacterial species. Cole and Wright concluded that the studies of the investigators just mentioned "have emphasized what was already known from qualitative studies, that within recognized species there are distinct cultural races or varieties, each with its own characteristics and range of variability, and that these may exist side by side, independent of the environmental conditions." They further cite a statement made by Winslow in 1908, that "bacterial types (species) should never be described on the strength of an examination of a single individual strain, but only after a comparative study of the numerical frequency of each particular character in a considerable series of cultures." Although he prefers to view the variations shown by any particular species of bacteria as primarily associated with its cyclic development, nevertheless Hadley (22) has not entirely overlooked the possibilities of racial or varietal development within a

species. He says: "It is perhaps true that, of all the variations shown by different strains, many are due to the isolation of biotypes, each possessing its own range of variation, and probably overlapping other ranges."

#### MORPHOLOGICAL VARIATIONS

In an address delivered in 1924 before the Mycological Section of the American Botanical Society, at the Cincinnati meeting, entitled "Some Evidence for Gonidia Formation in Certain Bacteria," the writer showed a relatively large number of lantern slides, representing micro-photographs depicting morphological variations within a bacterial species. In the meantime there have appeared a number of articles and books (2, 32, 33, 34), some of which show figures quite comparable to those that the writer has found. These authors without exception have interpreted the various forms shown by any one species as indicating complexities in the life cycle which had not been previously recognized in an adequate fashion. Gonidia, buds, sex organs, zygospores, coccus and rod forms, mycelioid and single-celled individuals, have been envisioned in species that have in the past been considered as monomorphic. The work of Mellon is particularly worthy of attention. In a series of papers he has brought to light some interesting data concerning the morphological and cultural variations in a number of bacteria. He isolated a strain of *B. coli* from a patient suffering from pyelitis which "grew with such pleomorphism as to suggest a fungus." It had long filaments, some of which were branched, and many very large coccus-like forms, which developed from the filaments. For staining he used carbol-thionin with which "fixation artifacts are . . . avoided." The interesting thing about this strain was that the pleomorphism quickly disappeared when the patient no longer received urotropin and only normal-looking *B. coli* were to be found. But when the drug was again administered the fungoid filaments immediately reappeared. Grown on Endo agar at 37° C. from a single cell isolation, the plate colonies which developed showed the fungoid form of the organism. Preparations of such colonies showed a peculiar arching or tilting of two adjoining cells of a filament resulting in an apparent union of the arched

portions and giving rise to a globoid structure at the point of union. It is these roundish bodies which he takes to be zygo-spores. While his micro-photographs do not reveal the details of the union nor of the cytological phenomena which may be involved, nevertheless they present an almost convincing demonstration of a union of two cells. Assuming that this is a sexual act, the term "zygospore" which Mellon applies is to be questioned, although the photographs seemingly show gametes of equal size. But to compare this with the sexual process in yeasts, as Mellon does, is to overlook the subsequent development of a rather highly developed fungus with its asci and ascospores, although, superficially at least, the apparent union itself is very comparable to that which Mellon finds in this bacillus.

The closest approach of these "zygospores" to any fungus structure which the writer has found is that shown by Zopf (41) and Dangeard (7) in the Ancylistales, a group of parasitic water-molds recognized by mycologists as representing very simple forms of fungi. In the genus *Myzocyitium*, Zopf and Dangeard have each presented drawings of forms in which adjoining cells of a relatively simple thallus may function as sex organs which give rise to an oöspore. Likewise in *Lagenidium*, also representing a very low form of fungus body, adjoining cells of a filament may send out side branches which function as sexual bodies, one as an antheridium, the other as an oögonium. The roundish oöspore is the result of the fusion of these two organs. These are merely suggestions of comparable structures which are to be found in the fungi; but so little is known about the morphology and cytology of the bacterial bodies under discussion that it is very difficult to homologize with any degree of certainty. Aside from the proper designation of these bodies, Mellon has revealed some interesting data about them. They take the form of giant cocci and he regards the arthrospores of the older bacteriological literature as comparable to them physiologically. They are usually to be observed only "under conditions of marked aërobiosis such as pellicle formation and on agar slants." In another article (33) he presents the subsequent history of these "zygospores." When a transfer is made from a mucoid colony which gives rise to these roundish bodies, the resulting colony on an agar slant shows only

large coccoidal forms in the condensation water. "Since none of the small, normal-sized (rod-shaped) *B. coli* were present it seems quite clear that these mucoid colonies represented a reproduction of the larger coccoid forms whose immediate antecedents were the zygo-spores. . . . " When these "zygo-spores" were inoculated into broth under the warm stage they were observed to reproduce as enormous coccoids but when inoculated into agar they reproduced not as coccoids but as filaments or rods, which in turn gave rise to zygo-spores. In still another article (34) he describes similar structures with comparable life histories in a diphtheroid strain of bacteria. Likewise, he now presents additional studies on the morphology of *B. coli*, with micro-photographs showing gonidia or buds. By staining intravitaly he observed unipolar and bipolar buds or gonidia being formed on the rods and in some cases these buds gave rise to secondary ones. These resemble somewhat the figures shown by the present writer (40) for bud formation in *B. tumefaciens*, and are also comparable to figures which will be here shown and presently discussed.

While using a bacterial flagella stain, involving a method which has not yet been described, certain peculiar particles were noted which appeared quite frequently in young cultures (PLATE 29) of an organism which is closely related to the ordinary potato bacillus, *B. mesentericus*; the organism is a relatively large, peritrichous, spore-forming rod and, whatever may be its exact identity, it belongs to the group of which the hay bacillus, *B. subtilis*, is the type. It was isolated from a plug of a potato tuber, the tuber having been derived from a plant severely diseased with Irish potato mosaic. As the same organism appeared in several tubes from such plugs, while it was absent in similar tubes in which plugs of disease-free tubers were used, there appeared the possibility that it might be associated with mosaic. However, inoculation experiments were all failures and there is no good reason for believing that the organism is not an ordinary soil inhabitant which, on account of its resistant spores, remained viable and adhered to the surface of some tubers, even after an hour's immersion in a 1 to 1,000 mercuric chloride solution. The particles just mentioned were to be found in both young and old cultures and on various nutrient media.

The frequency with which flagella were to be found attached to these particles seemed surprising (PLATES 29, 30), for, as is well known, flagella are readily lost or cast off from normal rods and, if these particles represent dead or dying bacteria, it is rather odd to find the flagella still adhering to the decomposing material. But what appeared most surprising was the relatively large numbers of apparently disintegrating rods and of particles which were undoubtedly remnants of rods (PLATES 29, 30). Some rods may be seen to be stained deeply with the interiors appearing homogeneous and others may be seen as lighter staining structures with markedly differentiated portions. It is the last-named rods which show particles or granules of various types appearing within the contours of the rod and not infrequently a flagellum may be seen arising from or oriented toward such particles (see PLATES 29, 30). Frequently the rods have disintegrated to such an extent that their outlines have disappeared and the form of the fragments may not lead one to suspect their relationship to the usual rod-shaped figure. But a careful study of many preparations, both in living, hanging-drop cultures and in stained material, leaves no doubt of the relationship of these particles to the rods. They are not artifacts and they are not contaminations. I have studied this organism for more than five years and have made repeated transfers from single colonies and the preparations are consistent throughout.

As to the particles representing remnants or dissociation products of rods there are a number of interesting features associated with them. It has already been noted that flagella are frequently to be found attached to such particles, even when they are free from the mother rod (PLATES 31, 32). These particles or granules vary greatly in size and in shape, some being so tiny that were it not for the subtending flagellum they would be either overlooked or would not be resolved with the highest power of the microscope. The writer has attempted to measure some of the smallest and finds that it is hardly possible to do so even with a Zeiss filar micrometer. They measure less than  $0.1\ \mu$  and, taking into consideration the increase in size that one expects in mordanting and in depositing a stain, the actual size would be closer to  $0.05\ \mu$  than to  $0.1\ \mu$ , or  $50.0\ \mu\mu$ . It is interesting to compare such sizes



with those given by Duggar and Karrer (8) for particles constituting the "contagium vivum fluidum" of the mosaic disease of tobacco. They find that these disease-producing particles are approximately the size of colloidal hemoglobin, which is stated to be between 30 and 40  $\mu\mu$ . Thus, aside from any consideration as to the biological significance of the particles under discussion there can be no doubt that they approach the sizes of a filterable virus and yet are observable. Grading upward in size from these minute particles, there are to be found many others which are larger, often to be observed clumped more or less together, some being good-sized portions of a fully developed rod. In shape they vary from a more or less regular roundish, oval, oblong, or cylindrical figure with terete walls to a somewhat irregular structure with no well-defined outline. They stain very readily and heavily in contrast to the walls of the mother rods which stain but lightly in the same staining period, even with carbol-fuchsin. Similar particles have been observed within the rods and free from them in a number of species, though the number of the particles has not been nearly as great as in the species previously mentioned. Among those showing such granules are the following authentic species, *B. typhi* (*B. typhosus*), *B. vulgatus*, *B. mesentericus*, *B. subtilis*, and *Proteus vulgaris* (PLATES 30, 33).

The question which presents itself for consideration with reference to these particles is, are they living entities or merely dead, disintegration products? Those who are acquainted with Löhnis' own work (29, 30) and with the splendid survey of the literature in this field which he has presented (28) will recognize in the microphotographs here presented a striking resemblance to many of the figures that he has brought together. In a relatively large number of bacteria he has found certain phases which seemingly indicate a far more complex life history than has been assumed to exist. In this paper we are primarily concerned with those phases which Löhnis interprets as buds, granules or gonidia which represent distinct parts of the life cycle and which he believes are living forms going through a more or less specified developmental process and capable of giving rise to the normally accepted rod or coccus inherent to the respective species. Indeed, it may not be too much to say that from one point of view the figures here shown

add materially to his interpretations and that in very few, if in any, figures which he presents does one see such good pictorial evidence of a bud or gonidial forming process. But are these granules, with their flagella, really alive and how can this be proved?

Aside from the morphological evidence observed in stained preparations there are at least three possible ways of determining the vitality of these granules. First and perhaps most important would be to attempt direct microscopic observations of living preparations; second, to attempt to separate the particles from the large rods by using a bacteriological filter and noting any colony growth which may develop from the filtrate; and third, to attempt to isolate single particles and to determine their ability to grow or to reproduce. All three of these methods have been tried by the writer, the last one only to a limited extent because of the great difficulties involved and the first two in a rather extensive series of microscopic observations and filtrations. The results, which are now to be presented, are not convincing and the writer must say immediately that the evidence now at hand for the belief that the particles are alive is not as good as the writer considered it to be four years ago, although the evidence at that time was not taken to be conclusive.

As to the work dealing with direct microscopic observations, use was made of the finest series of apochromatic lenses available, with various sources of light and with different magnifications, including the highest to be obtained. As the organism grows very rapidly at 25 to 30° C., most of the microscopic work was done in a small transfer chamber where the temperature fluctuated but slightly and approximated or reached the optimum for colony development. Transfers from both young and old cultures were made to sterile cover glasses, the medium being either a three mm. loopful of nutrient broth or of the water of synaeresis from the base of a nutrient agar slant. These were then used as hanging-drop slides in Van Tieghem cells. The rods being relatively large, 0.75 to 1.0  $\mu$  by 2.5 to 7.0  $\mu$ , were readily observable under the microscope even with 16 mm. objectives and low-powered oculars. The specific objects sought for were fragmented rods or particles which showed true motility or direct ocular evidence

of increase in size or of reproduction of any observable particle, whether it be large or small. That the medium and other environmental factors were suitable for growth in these cells was ascertained by noting the relative number of individuals within the drop after a given length of time. In addition to noting the free particles considerable time was spent in observing the fragmenting rods which still contained one or more granules and in which the original contour of the rod was still to be seen. Briefly, it may be stated that, as a result of making hundreds of such observations, many of which involved continuous staring down the barrel of the microscope for several hours at a stretch with but brief periods of intermission, in not a single case was there clear-cut evidence of increase in size in any observed particle nor was there any evidence of multiplication other than by the ordinary division of the unfragmented rods. As the stained preparations showed these particles to be frequently possessed of flagella, indicating motility, if they were alive, then in living mounts they should move about. While no difficulty whatever was experienced in observing some of the particles and watching them closely for signs of motion, other than showing the usual Brownian movement, only two particles in different cases were seen to be definitely in motion, both of them being within the confines of the old mother-rod wall. When first observed the writer felt that the evidence was complete; one could see the particle in each instance rapidly darting about in a space of about 1.5 by 5.0  $\mu$ , impinging first against one end of the old rod wall and then against the other. But, somewhat later while examining a stained preparation mounted in water, one in which osmic acid and carbol-fuchsin were used in the different steps of mordanting and staining, the same sort of motility was observed with even clearer definition. The old rod-wall was lightly stained and the very deeply stained particle could be seen darting back and forth. It is very difficult to believe that this particle was alive and it seems preferable to consider the motion as being the result of some physical process, either in the form of diffusion or osmotic currents of unequal intensity in different parts of the rod, or of irregular temperature expansions and contractions, or of some electrical phenomenon. But, whatever the explanation

may be for this movement, it seems more reasonable to assume that the particle was not alive in this stained preparation and consequently one is forced to conclude that the vitality of the moving particles of the living preparations is open to question. As far as the evidence from direct microscopic observations of living material is concerned it must be conceded that it falls short, to say the least, of proving that the particles were alive.

The next line of evidence to be considered consists of a relatively large number of ultra-filtration experiments. Five different types of bacteriological filters were used, three of which did not permit the passage of dextrin in a one per cent solution, as indicated by the iodine test, and the other two withheld a fresh, one per cent, defibrinated, hemoglobin solution and permitted the passage of only a part of the dextrin. Twenty-four attempts were made to filter young and old nutrient broth cultures, precautions being taken in each case to guard against contaminations. Twelve of these yielded pure cultures of the organism filtered, after a 24 to 48 hours incubation period, nine filtrates remained sterile, and three were thrown out of consideration because transfers from the filtrates did not yield uniform pure cultures and hence were open to the objection that they may represent contaminations. Only the two filters which permitted the passage of a part of the dextrin enabled the organism to pass through the pores. The evidence from these filtration experiments for the passage of some of the particles and their ability to reproduce typical colonies certainly appears substantial. The fact that each of the filters was carefully standardized by means of colloidal hemoglobin and dextrin solutions and that those filters which permitted the passage of the organism possessed pores which partly withhold even part of the dextrin solution, would seemingly indicate that the unfragmented rods could by no conceivable means have passed through the filters. The rods, as previously noted, are exceptionally large, many of them being fully  $1.0\ \mu$  in the smallest diameter and none are less than around  $0.75\ \mu$ . If such rods were able to pass through a bacteriological filter under ordinary methods of filtration, using a simple vacuum pump operated from a hydrant, for relatively short periods of time, what would be the chance of obtaining sterile filtrates of any microorganism?

*B. prodigiosus*, as Mudd (35) points out, is almost a classical example of an organism that does not pass through ordinary bacteriological filters and yet this microbe, as given in Bergey's manual (4), measures only 0.5 by 0.5 to 1.0  $\mu$ . Furthermore, as a check on the method of filtration, sterile nutrient broth at different times was passed through the filters and the transfers from the filtrates were invariably sterile. Likewise, the use of hemoglobin and dextrin solutions for standardization purposes should constitute a far more rigid check on the fineness of a filter than the use of any known microorganism, including the smallest. According to Duggar and Karrer (8) and Duggar and Armstrong (9) who used these substances in standardizing filters and thus determined the sizes of the particles of the virus causing the mosaic disease of tobacco, colloidal hemoglobin particles measure approximately 30  $\mu\mu$ , and dextrin particles measure even less than that. When filters will withhold the passage of colloidal hemoglobin measuring 30  $\mu\mu$ , it is almost inconceivable that the same filters would permit an organism to pass through which is at least 25 times as large in the smallest diameter. But, in view of recent work (35, 36) indicating that a microbe may or may not pass through a filter, depending upon its motility, upon the electric charge, and upon other factors unrelated to the size of the bacterium, what bacteriologist would be willing to stake his reputation on such filtration experiments as offering conclusive proof for the passage of viable particles and not of unfragmented rods?

The third line of evidence to be considered for the viability of the particles involves the isolation of single particles and of fragmented rods and of observing their ability to produce growth. The difficulties inherent to such a procedure may readily be imagined. It may be of interest to record the manner in which this was attempted. The work was conducted in a completely enclosed transfer chamber which had been heavily steamed and the microscope to be used was thoroughly cleaned with a cloth moistened with a disinfectant. A young culture was so diluted with sterile water that a one-half mm. loopful examined in a hanging drop showed by direct microscopic observation only one or two whole, unfragmented rods, or fragments of

rods. The hanging drops were made on sterilized cover slips which were placed over a sterilized, grooved slide. A direct count of bacteria was thus made of each drop and the slide, handled with sterile forceps, was then placed, drop downwards, on a plate containing nutrient agar or nutrient broth. The greatest difficulty in this method resulted from the necessity of adding sterile water to the edge of the cover slip in order to prevent drying out of the small, hanging drop. The method works very well indeed for picking up rather small fungus spores where lactic acid may be added to the edge of a cover slip to prevent drying and this acid prevents bacterial contaminations. But in the case of this bacterium the lactic acid prevented growth, and when the acid was not used contaminations were discouragingly frequent. Nevertheless a few fragmented rods were successfully isolated and in every case the transferred particles failed to produce a colony. Single, unfragmented rods, on the other hand, produced normal colonies. There is of course the possibility that while some particles may be dead others are alive and that live ones were not present among those which happened to have been transplanted. Although a great deal of time was spent in this sort of work, the evidence appears to the writer to be uncertain, indicating, if anything, that the fragments are not alive. It thus appears that of the three lines of investigation undertaken to determine the biological significance of the fragments, only the filtration experiments lend any appreciable support to the idea that the particles are living and capable of producing growth.

If these particles are not alive, then what does this phenomenon represent? Particles or granules have been observed by various investigators in organisms closely related to the one under discussion. In *B. mesentericus*, *B. subtilis*, and *B. mycoides*, Amato (1) has observed and figured certain granules as occurring within the rods. These he believes to be nuclei which go through a certain cycle. From germinating spores there originate, according to his conception, rods with a single, limited, central body. This divides into two and is followed later on by a regular cell division, giving rise to two daughter cells. Following this the central body in each daughter cell disintegrates into very fine granules followed again by cross-wall formation. The granules

arrange themselves along the periphery of the rod and then coalesce into a heavy-staining, thick, spherical body. This body, with the evacuation of some chromatic substance, which passes toward the poles, eventually becomes the spore. As Amato makes no mention of disintegrating rods and free particles prior to spore formation, there is no tangible basis for comparison with the granules under discussion, although as far as granules within undisintegrated rods are concerned, there are certain points of similarity, notably a peripheral arrangement of particles, which are occasionally to be observed (PLATE 31). Fuhrman (18) figures a granulation process as occurring simultaneously with a loss of flagella in *B. subtilis* prior to spore formation. But he, also, fails to show any disintegration of the rods prior to spore formation, nor does he indicate a disintegration which is not connected with spore formation. Quite recently Andervont and Simon (2) in studying certain pits that developed in colonies of *B. cereus*, growing upon agar slants, found that the pits were the result of a disintegration process of the non-spore-forming rods. Many of these presented a variable number of rounded projections along the lateral surfaces while "others appeared as mere shells containing granules of variable size and number." They also observed various sized granules free from rods and decided that they represented the contents of rods that had disintegrated. Pit formation was then concluded to be essentially brought about by the disintegration and liberation of the cell contents. They present figures which, while small in size and few in number, show striking resemblances to those here presented and while no pits or "pellucid" areas were observed in the cultures of the organism here studied, nevertheless the process of disintegration appears quite similar, and the organisms, being large, peritrichous spore-formers, are certainly rather closely related. It is also interesting to note that out of four trials they were able to obtain two filtrates through Berkefeld V and Berkefeld N candles which after a week's incubation gave rise to growths that were identical with the original culture and they suggest that the disintegration process may be interpreted "in the sense of Löhnis' symplastic hypothesis, in which case one would expect that a regeneration of the organism from the gran-

ules could be effected." This naturally leads us to a consideration of Löhnis' work. Briefly it may be stated that, as a result of his work and that of his associate (29, 30) largely on various species of *Azotobacter*, a number of clear-cut forms have been recognized within a species; among these are the following: first, a roundish coccoid form, second, a rod-like form, third, a form in which the coccoids and rods show granules or gonidia, occasionally taking the form of buds or branches capable of reproducing as such or of giving rise to the parent form, fourth, an amorphous form or symplastic stage in which the contents of coccoids and rods have fused into a more or less irregular mass free from definite cellular structure and possessing the power of producing living granules or regenerative units, fifth, a form resulting from a union of two or more cells, and sixth, a fungoid form of more or less irregular, large, bladder-like or hyphal cells. It is important to note that they have concluded that all species of bacteria have a symplastic stage and possess other stages comparable to those which they observed in *Azotobacter*. Löhnis and Smith's observations have in part been confirmed by Jones (26). He also finds in *Azotobacter* that individual cells "may develop reproductive granules or gonidia within their cell plasm, which on disintegration of the mother cell are dispersed, increase in size, become typical *Azotobacter* short rods, ovals or spheres, and reproduce by fission. The young cells are motile. The reproductive granules vary in size, some being very minute." Jones was unable to filter these through a Berkefeld filter. In addition to the gonidia, he is also able to observe the form that Löhnis and Smith have designed as the "symplastic stage" and the regenerative granules which arise from the symplasm. "On emergence from the 'symplasm' these granules grow to young *Azotobacter* cells and reproduce by fission." Just what evidence he used for convincing himself that the granules grew is not stated but it is presumable that the appearance of stained preparations was the criterion. As to the large, irregular, involution (?) forms that Löhnis in part designates as fungoid, Jones also was able to find these, but, contrary to Löhnis and Smith, he finds them to multiply only to a very limited extent, the multiplication being in the form of a budding process. He studied these



in a hanging block culture and "in only a very small percentage of cases did the involution forms show any tendency to develop or reproduce." Finally, he is unable to confirm Löhnis and Smith's findings of spores or of any true conjugation. Jones did not observe the gonidia or the symplastic stage in species of bacteria other than in *Azotobacter*, although, judging from his statements, one may infer that he looked for them in other microbes. One is forced to conclude that Jones' confirmations of Löhnis' work are very slender.

It here becomes desirable to undertake an analysis of Löhnis' work and the proofs that he has presented for the viability of the forms, enumerated previously, that he has found within a species of *Azotobacter* and which he believes are also to be found in all species of bacteria. First of all it should be stated unequivocally that the forms he pictures are not contaminations or artifacts, for the writer's own studies of living, unstained preparations as well as the stained preparations show practically all the types that Löhnis has found and those here shown are in a species quite unrelated to *Azotobacter*. In the days of Alfred Fischer it was perhaps necessary to emphasize the common occurrence of plasmolysis, plasmoptysis and other artifacts in stained preparations, as well as the necessity of guarding against contaminations, but it is hoped that bacteriological knowledge and technic have made some advance since that time. While Löhnis may have had some contaminations in the hundreds of cultures which he worked with, it would be ridiculous to assume that any large part of his work is based on contaminations. It is more reasonable to assume that his cultures were largely pure and that the forms he found belong to the species to which he ascribed them. But, granting this, are his interpretations of the functions of these forms and the role they play in the life cycle open to question? His evidence may roughly be divided into two classes: one, microscopic studies, largely of stained preparations, and two, filtration experiments. As far as his published micro-photographs are concerned, much additional evidence may still be desired. This is particularly true for figures which would clearly show the transition of one stage to another, for example, gonidia giving rise to other forms, or symplasm forming reproductive

bodies. Figures showing arthrospores, cysts, and endospore-formation are far from clear. The photographs as a whole are not convincing. Concerning his filtration experiments, he attempted to pass a number of organisms, which showed gonidia, through Chamberland bougies and was able to see the small gonidia by the use of the dark field, "some of them being actively motile." Large bodies were not observed. The filtrates, when transferred to various media, all produced a very scant, thin, slimy growth, and microscopic observations showed in no case any large forms developing which would be comparable to the normal vegetative forms of the bacteria. Stained preparations of these transplants showed "gonidia germinating to minute rods" and dark-field studies showed "clearly that the filterable gonidia also form a symplasm in the same manner as the larger ones, which in its turn produces new small cells." His photograph of this symplasm is not especially self-revealing; it shows simply a clump of minute particles. When small quantities of filtrates of *B. subtilis*, *Bact. pneumoniae*, and *Bact. fluorescens* were transferred into ammonium-citrate solution, a "quick regeneration took place," with sediment formation in two days' time. Stained preparations of these showed "many pale, stained, small granules and minute rods, . . . and also larger dark stained oval forms 0.5 to 1  $\mu$  broad, 0.75 to 1.5  $\mu$  long. These forms still differ considerably in their appearance from the normal rods of *B. subtilis* and *Bact. fluorescens*." "That they will turn back entirely to the normal large vegetative cells is not doubted, *but this still remains to be tested experimentally*." The writer has italicized the last clause because this seems to him as representative of the uncertainty of this filtration work. Summarizing the evidence that Löhnis and Smith have presented on the viability of the different forms and their relationship to each other, it may be stated that it falls short of proving their contentions. Their work, and Löhnis' splendid effort of bringing together the literature on the subject, has been very stimulating and may eventually lead to a far clearer understanding of the developmental possibilities within a bacterial species. That the evidence up to date is nearly as complete as some bacteriologists consider it to be (22) is very doubtful. For the present the writer is inclined

to believe that most, if not all, of the granules here described and illustrated represent a disintegration process of cells that are about to die and that this death is merely the outcome of autolytic reactions occurring sooner or later in all cultures of microorganisms. He further believes that Löhnis' gonidia and symplastic stages may be placed in the same category but he reserves judgment on certain large, irregular forms, frequently designated as involution stages representing the "lame and the halt" among the bacterial populations. More will be said of these later on.

The next feature of interest that is occasionally observed in stained and in unstained preparations of the organism isolated from potato plugs consists of certain large spiral or whip-like structures (PLATES 32, 33). They are to be observed quite frequently in mounts from the syneraesis water at the base of nutrient agar slants when the bacteria are actively motile and when stained preparations show large numbers of flagella. The writer believes these to be abnormally large flagella representing a group that have fused together and comparable to the teratological specimens that one observes in higher plants and animals, consisting of a fusion of tissues and organs giving rise to various forms of monsters. As observed in stained preparations they vary considerably in size and particularly in shape. Often they appear as rather thin spirals, though always appearing much thicker than normal or average flagella, of equal diameter and tapering but slightly at one or both ends. In this form they bear a very close resemblance to spirochaetes. At other times they take the form of wavy spindles, appearing very thick and heavy towards the middle and gradually tapering down towards the ends, simulating an organized symmetrical body comparable to those seen in the genus *Spirillum*. Then again they may be observed as giant whips taking the form of irregular spirals, being very thick at one end and gradually tapering towards the other end. Usually they are seen unattached to any rod but occasionally they give the appearance of being attached to a rod close to a pole (see PLATE 33). Various investigators have at times observed similar structures and have interpreted them to be clumps of flagella. Gins (20) observed them in *B. typhi* and *paratyphi* B. Novy (37) found them in condensation water of motile bacteria.

They occurred as single, large, spiral-shaped structures originating from the ends of rods or as "more complicated braided forms not unlike the strands of a rope, usually spindle shaped." Mellon (32) in commenting on Novy's observations of these structures concluded that "they were not flagella since they stained with simple dyes. . . . They were not motile." He considers them "the same as the chromatin skein" which he observed within the cells of *B. coli*. While there is the possibility that structures other than flagella may form spiral-like bodies, and Mellon's figures clearly indicate this, yet there can be very little doubt that the bodies described by Gins, by Novy, and those here presented are abnormal flagella, which may consist of single aberrant forms or of groups which have fused together. In view of their relatively enormous sizes it is not surprising that they stain with simple dyes and are non-motile. Indeed, the larger ones are clearly observable in unstained mounts, as previously indicated. The fact that quite a few of them have been observed in the organism under discussion, perhaps more than have previously been recorded as observed in any one species, does not necessarily mean that they are more common in this species than in others, particularly in other organisms where flagella are inclined to be large and present in relatively large numbers, but that the staining method utilized as well as the time spent in studying the morphology of this organism has presented exceptional opportunities of observing them. The writer has little doubt that the same sort of structures will in time be found in most, if not in all, motile bacterial cultures.

Various other forms have been observed in the organism obtained from the potato plugs, including a stage which is quite comparable to Löhnis' symplasm. What the writer has seen consisted of an irregular mass of material which, as a whole, stains rather lightly but with localized parts scattered irregularly through the mass taking a deeper stain. Sometimes these darker staining granules appear with a definite outline, either in the form of a minute roundish or oval-shaped structure, or as a small rod. Mostly, however, they are quite irregular or indefinite in outline, closely resembling the particles which result from the disintegration of the rods, and the writer is inclined to view the whole

structure as a mass of dead matter, in various stages of decomposition or disorganization, fortuitously clumped together, and representing the remnants of the fragmented particles. Sometimes parts of walls or the whole of old walls of rods are mixed in with the material, most of which is interpreted as being the cytoplasmic, storage, and nuclear material together with metabolic biproducts. Another stage to be observed in this organism consists of relatively large cells (PLATE 31, middle figure), usually clinging together in the form of filaments, many of them being bladder-like or spindle-shaped, while others are cylindrical. When seen unstained they closely resemble the hyphae of a fungus mycelium, the individual cells being filled with a compact, granular substance which gives the impression of being reserve food material. They take the anilin stains very readily and usually stain so deeply with carbol-fuchsin that no interior structures are observable. In width they are four to eight times the size of normal, vegetative rods. These mycelioid cells are commonly found in the sediment of old nutrient broth cultures, although they may occasionally be seen in young cultures of both broth and nutrient agar. They have been observed in all the organisms, previously mentioned, in which granules with attached flagella have been found. The writer ventures to predict that they will eventually be observed in most species of bacteria. They have already been observed and illustrated in quite a few species including *B. coli* (31, 33, 34), *B. anthracis* (27), *B. mallei* (5), *B. Chauvoei* (19), *Clostridium butyricum* (38), and in *Azotobacter* (29, fig. 20). Löhnis interprets some of these bodies to be spores (28, p. 65) and others gonidangia (p. 124, 125), that is, bodies capable of reproducing a number of gonidia. There is no reason to doubt that they have frequently been observed by bacteriologists and taken to be degenerate or involution forms. Whether this is always a true interpretation remains to be determined.

#### SUMMARY

In discussing variability in bacteria attention is called to the fact that conditions which made for so-called normal growth often preclude the possibility of obtaining other structures which may develop in the same species under more natural conditions.

It is pointed out that variations may occur not only because of different phases in a life cycle, each with its own set of forms and functions, but also because of racial or clonal differences within a species.

While using a flagella stain on a peritrichous, spore-forming organism, originally isolated from a mosaic infected Irish potato tuber, numerous stained particles were observed, and here pictured, in young and old cultures. These particles frequently possessed flagella.

Similar particles were found within the confines of regular rods, often with flagella subtended or oriented toward such particles.

They vary greatly in size and shape, the tiniest approaching the size of the particles constituting the "contagium vivum fluidum" of tobacco mosaic.

Similar particles, but not as abundant, have been observed in *B. typhi*, *B. vulgatus*, *B. mesentericus*, *B. subtilis*, and *Proteus vulgaris*.

Attempts are made to compare these to gonidia, buds, and reproductive granules described by Löhnis and others.

Three different lines of evidence are detailed for determining the viability of the particles, including direct microscopic observation of growth or reproduction, ultra-filtration experiments, and pure culture isolations of particles.

It is concluded that the evidence for viability is uncertain, the ultra-filtration work favoring the theory that the particles are alive while the other lines of evidence are mostly negative.

The writer is inclined to the belief that the particles represent a disintegration process in the course of ordinary autolytic changes.

Various types of spirals are described and figured, some being observable in unstained preparations that are taken to be abnormal flagella.

A description of giant, mycelioid cells is given and the bodies compared with those previously described in a large number of bacteria.

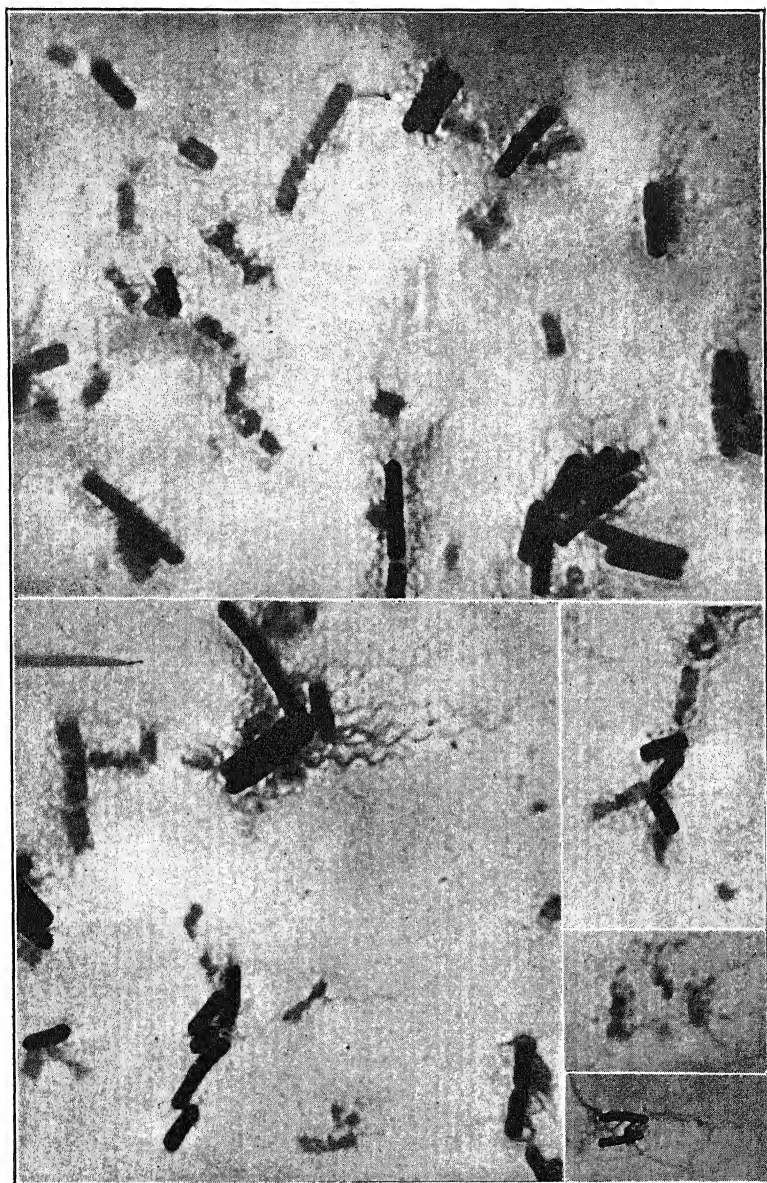
UNIVERSITY OF ARKANSAS,  
AGRICULTURAL EXPERIMENT STATION,  
FAYETTEVILLE, ARK.

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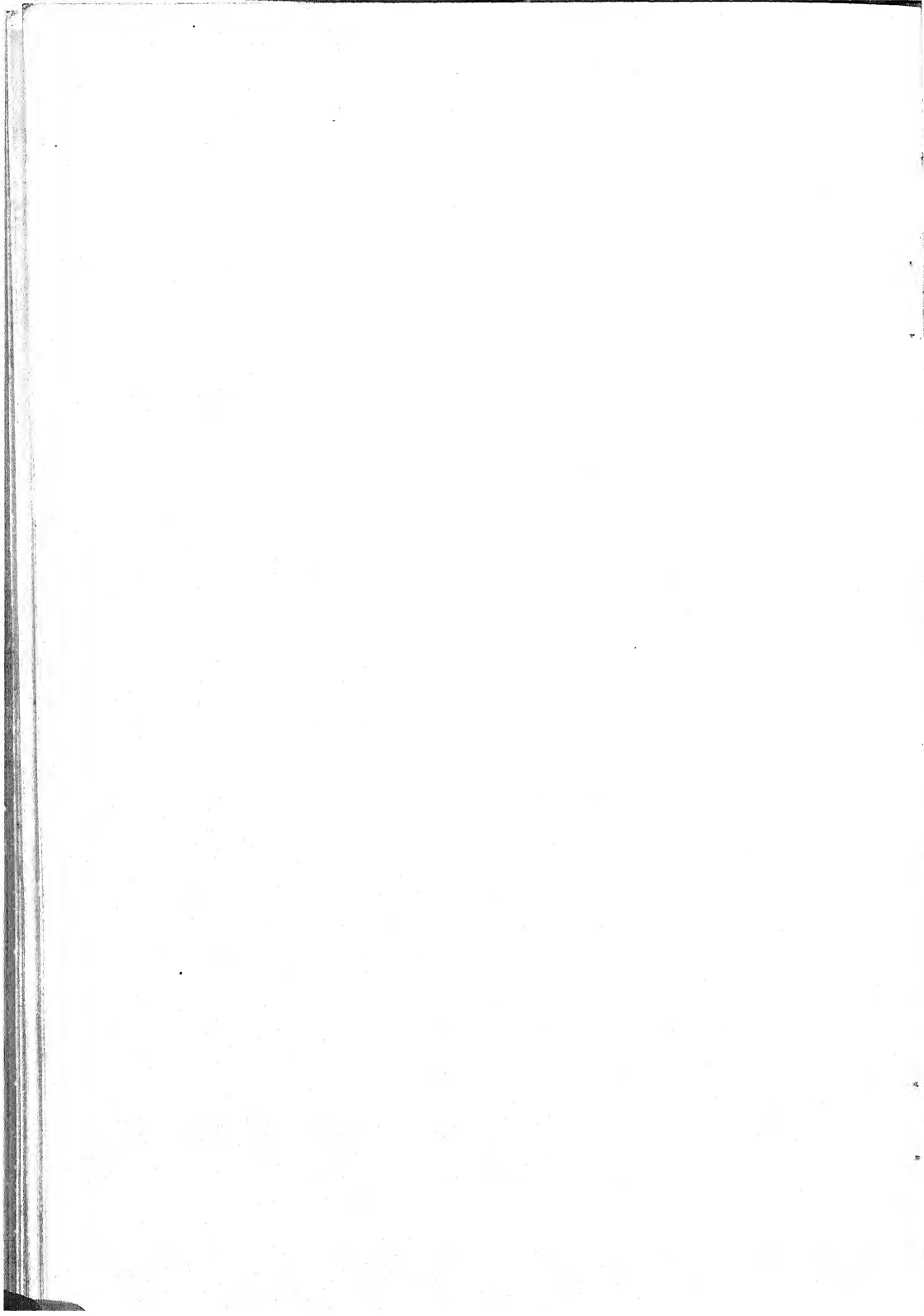
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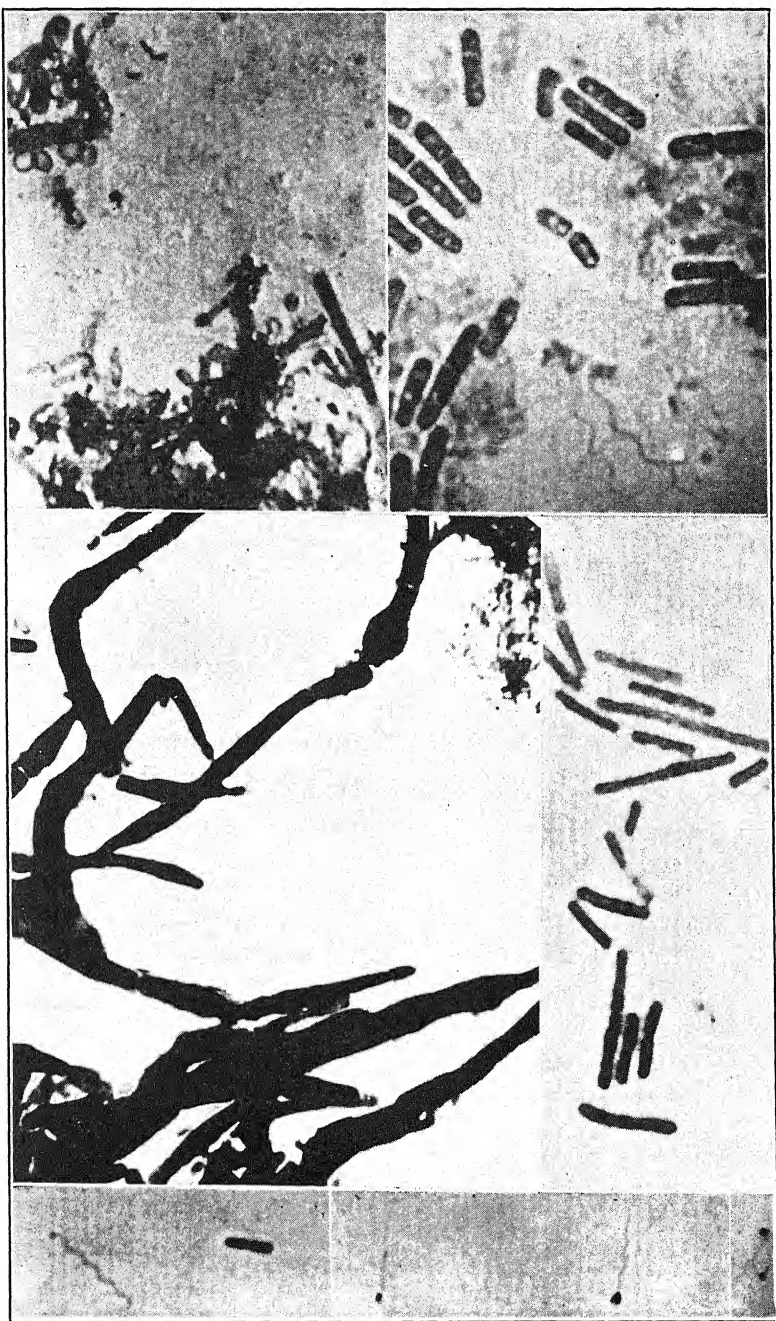
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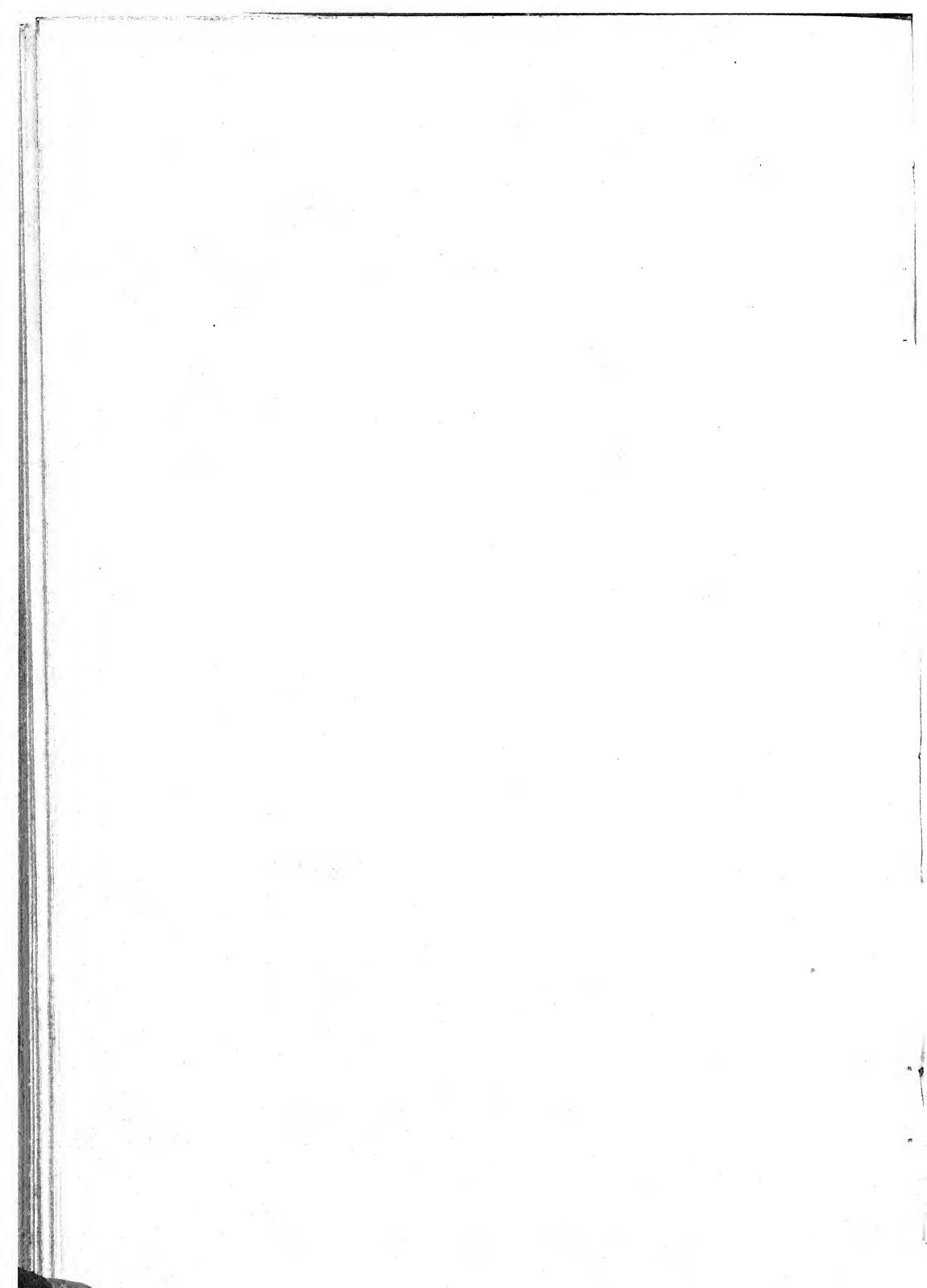


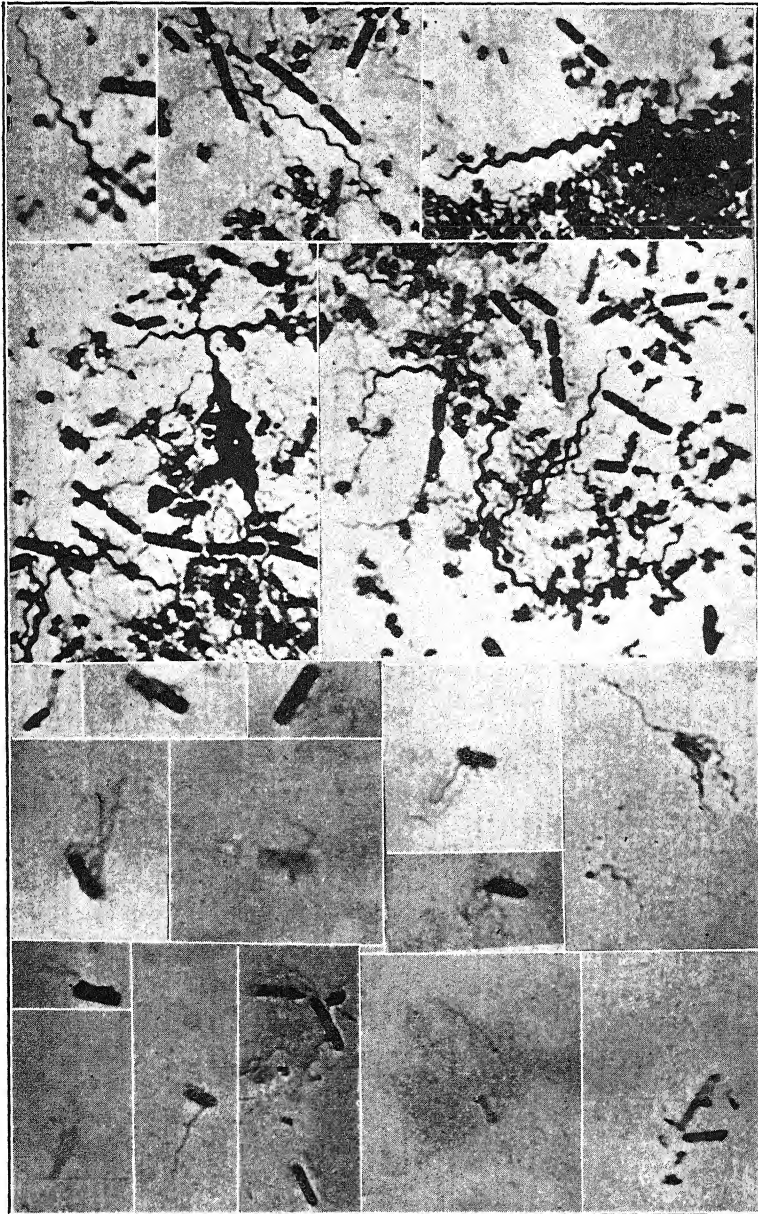
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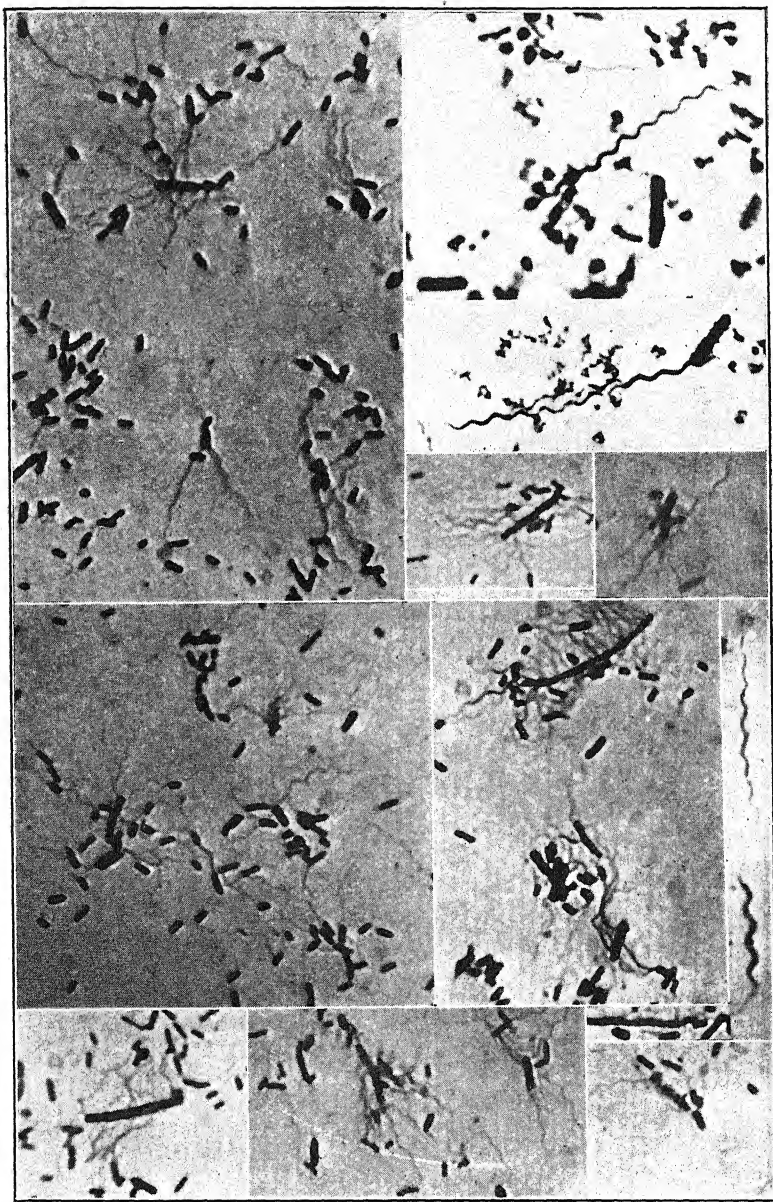
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VARIATION IN BACTERIA





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## EXPLANATION OF PLATES

### PLATE 29

Upper left-hand figure—colonies of the bacillus isolated from a mosaic diseased tuber, 48 hours old on nutrient agar. Spreading colonies near the glass; surface colonies smaller and with more of an irregular margin. Remaining figures showing fragmenting rods of this organism stained by the writer's flagella staining method. Magnified about 2500 times.

### PLATE 30

Rods of the organism isolated from a potato in various stages of disintegration; unfragmented ones are also seen, appearing homogeneous and heavily stained; magnified about 2500 times. Lowermost right-hand figure representing *B. typhi* showing a bud-like projection; magnified about 1500 times.

### PLATE 31

Upper right-hand figure showing rods of the potato bacillus with particles in the interior; note a disintegrating rod, toward the middle, with flagella concisely oriented toward two heavily staining particles.

Upper left-hand figure showing mostly remnants of old walls and residuum of interiors of rods, corresponding somewhat to Löhnis' symplasm.

Middle left-hand figure showing mycelioid types of cells of the same organism illustrated in Plates 29, 30 and 31.

Middle right-hand figure showing a localization of cell contents into a definite pattern in the rod toward the center, while surrounding rods are in the process of disintegration. Magnified about 2500 times.

Lower figures show free particles of the same bacillus, with subtending flagella.

### PLATE 32

Upper half of the plate shows particles observed within rods of the potato organism looking at times like buds and giving an irregular contour to the rods. Note also free particles with flagella attached. Some of these particles measure less than  $0.1\ \mu$ . Magnified about 2500 times.

Lower half of plate presents figures of spirals and giant whips stained by the writer's flagella staining method, taken to be abnormal flagella. Magnified about 1500 times.

### PLATE 33

Two upper right-hand figures as well as the two narrow middle right-hand figures show various types of spirals of the potato bacillus. Remaining figures represent pure, authentic cultures of *Proteus vulgaris* showing flagella and a granulation process, particularly in the large multiflagellated organisms; magnified about 1500 times.

## TEMPERATURE AND MOISTURE RELATIONS OF FOMES ROSEUS AND TRAMETES SUBROSEA

WALTER H. SNELL, W. G. HUTCHINSON, AND K. H. N. NEWTON

(WITH PLATE 34 AND 2 TEXT FIGURES)

Much of the material of this paper was put in form a few years ago as a contribution to the taxonomy of the two polypores with the rose-colored hymenium, *Fomes roseus* (Alb. and Schw.) Cooke and *Trametes subrosea* Weir. Until quite recently these two wood destroyers were commonly called *Fomes roseus*, with perhaps a mental reservation that there might really be two forms. Overholts in 1915 stated that the two forms were quite distinct, however (6, p. 68). The senior writer had become convinced of the same fact both from collecting these forms and from observations of cultural behavior in the laboratory and fructification in the field. In connection with various studies of structural-timber-destroying fungi, the senior writer became interested in means of identification of the cultures of wood-destroying fungi and especially in the thermal responses of the mycelium in culture as a feasible means of differentiating many of them (8, p. 24). With a view to adding material in substantiation of Overholts' separation of these two forms, work was done on various pairs of closely related polypores as well as these two, in order to find out the reliability of temperature responses as a differential. About the time that the material was ready for publication, Weir's paper (12) clearing up the situation with regard to these two fungi appeared and the pertinence of the temperature reaction studies lost its immediacy. In view of this fact, the material was withheld for the addition of more complete material on the moisture relations than was then at hand.

### VALUE OF THE TEMPERATURE FACTOR IN IDENTIFICATION OF CULTURES OF WOOD-DESTROYING FUNGI

It is often desirable to be able to determine the identity of wood-destroying fungi in the absence of fructifications. This identifica-

tion is possible in many cases because of the association of a definite and characteristic type of decay with the presence of a certain organism. There are, however, groups of wood-destroying fungi the individuals of which produce decays so nearly alike that it is difficult to distinguish them. This is true of the decays caused by some of the commoner forms, by many closely related species, and by many of the important structural-timber-destroying fungi. If, in addition to the absence of fructifications, certain distinctive formations such as strands, colored mycelium, etc., are not present, the only remaining method of determination is by means of cultures.

Falck in Europe (1 and 2) has done considerable work in distinguishing the decays of certain structural-timber-destroying fungi by means of such manifestations as mentioned, but has not developed the use of cultures for the same purposes. Cultural studies have received more attention in this country. For the purpose of distinguishing between species, even closely related ones, Long and Harsch (5) emphasize the advantages of color reactions obtainable upon a series of several agars selected because of their value in yielding differential color effects. The senior writer, in making a key of the cultural characters of five mill fungi (8, p. 24), used not only the type of growth upon a single agar,<sup>1</sup> color of the growth and presence or absence of the different kinds of secondary spores, but also the comparative rates of growth of the mycelium at certain temperatures.

This temperature reaction was found to be a valuable criterion in the distinguishing and determining of these fungi in culture. The test was successfully applied to certain cultures in the Forest Products Laboratory collection—cultures taken from decayed structural timbers in buildings in various parts of the country the identity of which were known, unknown, or uncertain. The temperature test was also applied to certain pairs of readily distinguishable but closely related species in order to find out if the difference in thermal response of the mycelium of the closely related fungi is the rule or the exception.

One pair of closely related polypores used was *Lenzites sepiaria* Fries and *Trametes protracta* Fries. They are usually considered

<sup>1</sup> Malt agar—3 per cent agar, 2½ per cent malt (Trommer's diastasic extract).

as separate species although it has been suggested more than once that *Trametes protracta* is only a poroid form of the former. Point is often given to this suggestion by the occasional occurrence of the two species or forms near together, even on the same piece of wood. The senior writer has found them together several times on bridge timbers. On one occasion, on a rough log bridge over a mountain stream, the senior writer found three of four poroid *Trametes protracta* forms occurring among a hundred or two lamellate *Lenzites sepiaria* fruit bodies. On another bridge of sawn timbers, the numbers of fruit bodies of the two species were more nearly even. Inasmuch as two pairs of cultures of each fungus (one pair of single spore cultures and one pair of tissue cultures) were at hand, a complete set of tests was run. The tests were made in triplicate and often repeated at all temperatures, upon agar from the same batch. The agar plates were poured from tubes containing 20 cc. of the medium. The plates to be used for the tests were inoculated with a 1 cm. square block taken from the growing border of a plate culture of the fungus, the square of inoculum being placed mycelium side down on the inoculated agar. The measurements recorded in Figure 1 are the results of averages of radial growth from the four sides of the inoculum square on the triplicate plates. Plates showing irregular growth for any reason were discarded, and the test was repeated. Very seldom, there was almost no growth from one side of the inoculum, whatever the reason might be, but as a rule the growths were very regular and there was almost no variation in the 12 measurements taken. Two points on the curves in the figures represent the extremes of variation.

It is to be noted from these tests that there is no apparent difference in the optima of the two species. *Trametes protracta*, like *Lenzites sepiaria*, has a comparatively high optimum temperature—between 30° C. and 34° C. Their upper limits of growth are, however, different. *Lenzites sepiaria* is not inhibited until after 40° C. is reached, while *Trametes protracta* was only barely growing at 38° C. and would not grow at all at 40° C. Also, the rates of growth of the two organisms are quite different. *Lenzites sepiaria* grows faster at all temperatures, the difference being more pronounced between 28° C. and 36° C.

There is a pronounced difference in the temperature reactions of the two organisms, not as to optimum but as to rate of growth, except at the lower temperatures. This fact supports the more commonly accepted view that *Trametes protracta* is a species distinct from *Lenzites sepiaria*. A test of growth upon a single agar at temperatures from 30° C. to 36° C. would serve to distinguish the fungi in culture.

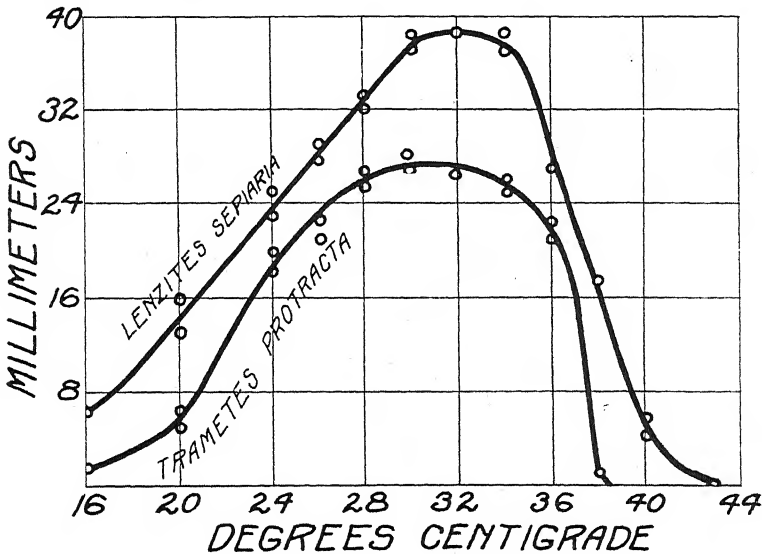


FIG. 1. Smoothed curves of the growth of the mycelium of *Lenzites sepiaria* and *Trametes protracta* on malt agar in relation to temperature. The pairs of circles at the different temperatures represent limits of variation of growth.

A second pair of species tried out in this way was *Polyporus abietinus* Dickson ex Fries and *Polyporus pargamensis* Fries. The results here were not as striking as with the preceding pair, but the two curves were sufficiently different in a number of places to provide a good differential on the basis of temperature, if a differentiation of the cultures of the two fungi should be desirable.

A third pair tried included *Polyporus resinosus* Schrader ex Fries and *Polyporus benzoinus* Wahl ex Fries. As a matter of convenience here, the two forms are considered to be separate species, although it is recognized that there is some dispute concerning the propriety of this arrangement. Many, including

Overholts (6, pp. 47 and 52), believe that the latter is only the coniferous wood form of the former species. Others, as for instance Lloyd (4, p. 334), think they are quite distinct and distinguishable. The senior writer has been interested in attempting to determine this matter. The most promising experiments embrace inoculations of hemlock, basswood and maple logs and bolts with cultures of each of the species, which have been made in two localities in the Adirondacks (experiments now 3 years old). The temperature test was applied also in order to see what it might contribute. Without presenting the data here, it may be said that the curves of growth are nearly the same for both fungi at most of the temperatures, but that there is a difference at 24° C. It may be added also that there tends to be greater irregularity in the growth of *Polyporus benzoinus* at 24° C. than in that of any other fungus at even the extreme temperatures.

This test was also applied in an earlier piece of work (9, p. 162) to demonstrate that the Agaric causing decay in cotton mill roofs is *Lentinus lepideus* Fries and not *Lentinus tigrinus* Fries. The growth of the latter at 30° C. was nearly double that of the former and the difference was useful in substantiating the less constant differences in appearance of cultures.

It is seen that the thermal responses of the mycelium offer reasonably definite means of separating the cultures of species that are ordinarily distinguishable on morphological grounds. This is shown by the positive results of the tests upon three sets of recognizable species, and further supported by lack of definite evidence in the tests upon *P. resinosus* and *P. benzoinus*, two forms about which there is more dispute from a mycological point of view, as they are at present separated chiefly on the basis of host.

With regard to the possibility of settling disputes as to the specificity of closely related forms, the foregoing experiments offer some encouragement, but no definite assurance. It is seen that well-defined species may react differently thermally. On the other hand, there seems to be no reason why they of necessity must. If *P. resinosus* and *P. benzoinus* are really two different species, the slight and irregular differences in their temperature curves give only slight evidence of the difference, very little upon

which to place any dependence. Leonian (3, p. 452) found that there was no specific difference in the rates of growth of the species of *Phytophthora*. While for the most part, heretofore, differences in physiological behavior have been taken only as evidence of varietal or racial difference, there is a growing tendency to depend upon functional response as a factor in taxonomy and in phylogeny. It would seem that organisms that live differently are inherently different, although difference in a single respect would hardly be sufficient ground for making species.

TEMPERATURE RELATIONS OF *Fomes roseus* (ALB. & SCHW.)

COOKE, *Trametes subrosea* WEIR AND *Trametes Feei* FRIES

Working originally with the idea that, if there was a definite difference in temperature response, it might indicate a difference of species and would at least support other evidence, a rather extensive set of tests was run with cultures of *Fomes roseus* and *Trametes subrosea*. In view of Weir's paper (loc. cit.) to the effect that there are distinct morphological differences between the two forms, the following results merely serve to substantiate his conclusions and to show the possibility of the use of similar methods with other species.

*Trametes Feei* was added upon Weir's suggestion merely as a matter of interest in connection with the entire problem and he very kindly sent a fruit body for culture purposes. Thus there are included the three polypores in the United States that have a rose-colored hymenium.

The cultures used were the following:

1. *Trametes subrosea*—single spore culture from tamarack, Wisconsin, 1916.
2. *Trametes subrosea*—single spore culture from *Prunus* sp., Rush Lake, Minn., 1917.
3. *Trametes subrosea*—single spore culture from spruce log, Crawfords, N. H., 1919.
4. *Trametes subrosea*—tissue culture from fruit body on spruce pulp bolt from Canada, 1921.
5. *Trametes subrosea*—tissue culture from fruit body on red oak fence post, Jackson, N. H., 1922.
6. *Trametes subrosea*—tissue culture from fruit body, Warrensburg, N. Y., 1923.
7. *Trametes subrosea*—single spore culture from same as 5.
8. *Fomes roseus*—tissue culture from fruit body on spruce beam, Jeffersonville, Vt., 1919.

9. *Fomes roseus*—tissue culture from fruit body on spruce beam, Jackson, N. H., 1922.
10. *Fomes roseus*—tissue culture from fruit body on hemlock plank, North Conway, N. H., 1922.
11. *Fomes roseus*—tissue culture from fruit body on Douglas fir down log, Big Basin, Calif., 1923. Sent by Dr. E. P. Meinecke.
12. *Fomes roseus*—tissue culture from spruce (?) beam on ground, Warrensburg, N. Y., 1924.
13. *Trametes Feei*—tissue culture from fruit body from Florida sent by Weir, 1924. Other data lacking.

The significant results are shown in Figure 2. Curves of all the cultures in the above list are not shown for various reasons.

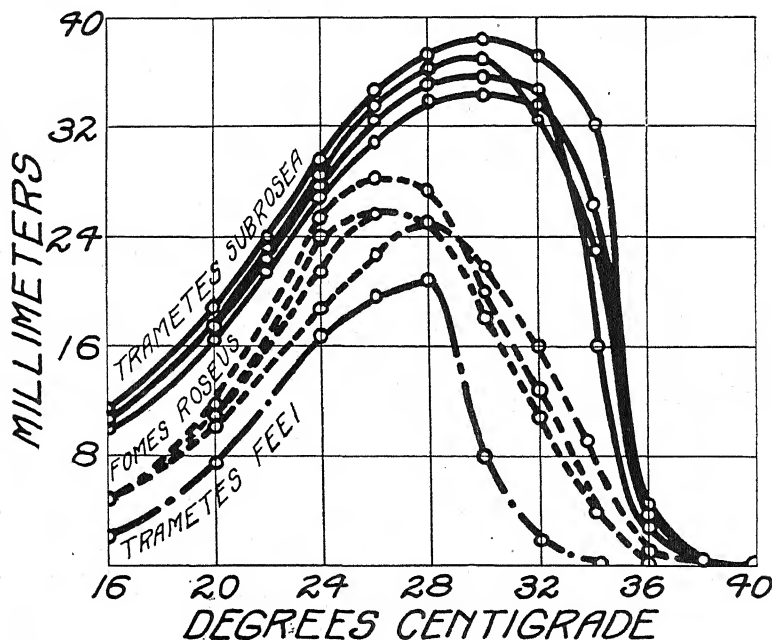


FIG. 2. Smoothed curves of the growth of the mycelium of *Trametes subrosea*, *Fomes roseus* and *Trametes Feei* on malt agar in relation to temperature.

The circles represent averages of radial growth from the inoculum block on triplicate plates, many times repeated. The cultures represented by the curves are as follows: *Trametes subrosea*, proceeding from the top down along the 32° C. line—1, two cultures from red oak (nos. 5 and 7 in Table I); 2, from spruce (no. 4); 3, from conifer (no. 6); 4, from cherry (no. 2); 5, from tamarack and spruce (nos. 1 and 3). *Fomes roseus*, from top down, along 24° C. line—1, from spruce (no. 9); 2, from spruce and hemlock (8 and 10); 3, from spruce (?) (no. 12); 4, from Douglas fir, California (no. 11).



In the first place, it was found that there was no difference in the curves of single spore and tissue cultures from the same fruit body. In the second place, there is no point in showing all the curves that fall in between the outside limits for a species, or in presenting pairs that nearly coincide. All the *Fomes roseus* curves are shown, but of those of *Trametes subrosea* only the more interesting are given. These include two curves of cultures of species growing on coniferous woods and two on deciduous hosts.

There is but one curve for *Trametes Feei*. Nothing is known about any variations within this species, but on the face of the only available result this species grows more slowly at all of the temperatures and is inhibited from growing at a lower temperature. It could be differentiated from the other two species if grown from 30° to 34° C.

Leaving aside for the moment the individual variations in the different cultures of *Fomes roseus* and *Trametes subrosea*, it is seen that there is a difference in the tendencies of the curves for the two species, from 26° C. to 36° C. A test at 30° C. or 32° C. apparently differentiates these two species with absolute reliability. The temperature test is the most reliable way of separating the cultures of the two species, because other characters of the growth upon agar vary so much. In color, the culture from spruce at Crawfords, N. H., is always pure white. The one from oak is a little pinkish (La France pink<sup>1</sup>), and the one from cherry is streaked with old rose or jasper pink, and may be somewhat Pompeian red with streaks of hazel. This latter culture was not much different in appearance from the *Fomes roseus* culture from California, although the other cultures of this species are decidedly dark colored—hazel to pecan brown or Rood's brown.

The consistency of the mycelial mats is likewise variable. The Crawfords culture of *Trametes subrosea* is decidedly "bunchy" tomentose like washed cotton flannel, and powdery when worked with a needle, while the New England *Fomes roseus* cultures form a very tough mycelial skin on the surface of the agar. The consistency of mycelial mats of the other cultures varies between these two extremes, the skin being tougher the more pronounced the color.

<sup>1</sup> Ridgway, Robert, Color Standards and Color Nomenclature, 1912.

An interesting example illustrates the reliability of the temperature test in differentiating the cultures of *Fomes roseus* and *Trametes subrosea*. In the earlier work, before many different cultures had been tried, Dr. Meinecke's specimen from California was welcomed as an opportunity to test the conclusions formed up to that time. The plant appeared without any doubt to be what was then called *Trametes carnea* by those who admitted of a difference in the two forms. As "*Trametes carnea*" it was tested. The first results were so disappointing as to appear to threaten the entire study. At 24° and 28°, the results not only did not coincide with what was expected of "*Trametes carnea*," but were even short of what had been obtained for *Fomes roseus*. The growth was from 3 to 8 mm. less at those temperatures than for *Fomes roseus*, whereas up to that time "*Trametes carnea*" had always given 2 to 8 mm. more of growth at those temperatures.

When the complete curve had been obtained, it was obvious that the plant from California was *Fomes roseus* and not "*Trametes carnea*" (*T. subrosea*). About this time, Weir's article already mentioned appeared, and while the writers were unable to satisfy themselves as to the identity of the fungus on the criteria given in the key, on the other hand Weir wrote of the specimen that he "unhesitatingly called it *Fomes roseus*."

At about the same time, it was noticed also in the cultures of *Trametes subrosea* that the one from oak had a consistently greater growth at most of the temperatures, and with very little variation in the different runs. In fact, this culture was found to respond more regularly than any other culture that has been tried. It was thereafter found that curves plotted for the various cultures of both species were different in some cases, although of course some curves coincided. The culture of the aforementioned species showing the least growth at most of the temperatures happens to be the one from another hardwood—the one obtained from the exposed heartwood of a living cherry tree. The cultures from what is considered the normal substrate for *Trametes subrosea*—wood of coniferous trees—gave a more nearly uniform response. The natural human tendency to endeavor to explain everything, even upon quite gratuitous assumptions, calls up two

suggestions in this connection: one, that the physiology of the plant has been changed slightly by the somewhat unusual food substrate—*i.e.*, angiospermous wood; the other, that there may be different strains or forms of the organism, some of which have become adapted to hardwood habitation.

There is no reason why there should not be strains of these wood-destroying fungi. Schmitz (7) has found what appear to be strains of *Fomes pinicola* Swendener ex Cooke. Further work to obtain data upon this point in connection with the fungi considered in this paper is contemplated.

OBSERVATIONS ON THE MOISTURE RELATIONS OF *Fomes roseus*  
AND *Trametes subrosea*

The senior writer has been interested for some years in various aspects of the moisture relations of some of the wood-destroying fungi and certain fungi producing cankers on woody plants (cf. 10 and 11). There is much of interest and of importance in the ecology of the structural-timber-destroying fungi. Moisture appears to be the determining factor in the occurrence of certain of these fungi. In connection with general observations on the two fungi under discussion over several years, it has been noted that *Fomes roseus* has been found with few exceptions upon hewn timbers more or less exposed to drying by sun or wind—at least in situations essentially not moist. On the other hand, *Trametes subrosea* has been collected on logs covered with bark or on wood in situations decidedly moist. The former has been located in fields, old sawmills and other buildings or very open woods; the latter in ravines, near brooks, waterfalls, etc., or if in open locations, well protected by grass, ferns or other plants.

In a pasture which was once the well-known Enchanted Woods (white pine) near North Conway, N. H., surrounded by younger pine growth, there was found a scattering of these two species of polypores that provided a chance for observation and experiment with regard to their moisture reactions. In the clearing there stood, up to recently, the framework of the old sawmill that cut up the pine of this beautiful spot. Among the various fungi found in this old sawmill were live sporophores of *Fomes roseus*, occurring on pine beams in some of the moister and more protected

places overhead, in the drier places on the framework and on the pine beams on the ground outside, exposed to sun and wind. There was no *Trametes subrosea* on the pine or hemlock wood of the structure at all, but it was found in abundance along with *Fomes roseus* in a pile of hemlock, spruce, and pine logs and bolts within a hundred feet of the sawmill. This pile of logs and bolts was backed up to the east side of a stand of sapling white pine, and surrounded with a dense growth of sweet fern which reached up 15 or 20 inches on the pile. Here there was a sharp separation in the occurrence of these two forms: *Fomes roseus* on worked timbers in the open and on decorticated bolts on the top of the pile—both situations well ventilated; and *Trametes subrosea* on unworked timber in moist log-pile conditions, down low and protected from drying by the ferns and grass. This sharp separation of habitat suggested a difference in moisture requirements or dryness tolerances of the two fungi, either with regard to vegetative growth within the wood or to fructification on the outside. It appeared that the habitat of *Fomes roseus* was drier both as to substrate and as to relative humidity of the atmosphere than that of *Trametes subrosea*. The timbers upon which the former fungus grew should have been much drier not only by reason of their more open situation exposed to ventilation, but also because most of them were barked and sawn or hewn, and therefore more readily dried after each wetting. On the other hand, the logs in the piles bearing the latter fungus, with or without the bark, not only dried out less rapidly, but were not so well ventilated. Whatever may be the effect of the sawing or hewing of timber upon the rapidity of its drying, the effect of the bark is definite.

No tests were made of the moisture content of the logs or bolts in the pile bearing the two fungi, or of the beams in the mill bearing only *Fomes roseus*, but it was of course obvious that the upper bolts in the pile bearing only this fungus were dry on the outside most of the time except shortly after rains, while the lower bolts and logs bearing *Trametes subrosea* were wet longer, if not all day at times. However, the moisture content of the interior of the logs without doubt varied with that of the exterior.

In order to gain information upon the difference in relative

humidity of the atmosphere in these two places where the two fungi fruited, many readings were taken during the summer of 1922 with a hygrometer, at different points in both the log-pile and the old sawmill. The readings were taken repeatedly at all times of the day and night, and under all conditions of weather. A few of the readings selected at random are given in Table I.

TABLE I

RELATIVE HUMIDITY CONDITIONS WHERE *Fomes roseus* AND *Trametes subrosea* FRUITED, NORTH CONWAY, N. H., 1922

Day and time	Weather	Relative humidities			
		Bottom of log-pile where <i>Tr. subrosea</i> fruited	Top of log-pile where <i>F. roseus</i> fruited, but no <i>Tr. subrosea</i>	Sawmill where <i>F. roseus</i> fruited	Beam outside of sawmill where <i>F. roseus</i> fruited
August					
4th— 5 P.M.	Sunny, windy	68%	58%	49-57%	49-50%
7th— 9 A.M.	Rain	93%	90%	90-93%	90-93%
7th—12 M.	Sun and wind after rain	84%	78%	82% (floor over-head soaked)	74%
9th—12 M.	After 2 days of rain	60%	50%	50% ( <i>F. roseus</i> making new growth)	48%
16th—all day <sup>1</sup>	Hot, bright, little wind	48-53%	41%	43-46%	39-41%
18th—all day <sup>1</sup>	Hot, bright, little wind	71%	67%	68%	66%
21st—all day <sup>1</sup>	Bright, cool, high wind	45-48%	39-40%	43-44%	35-38%
26th—all day <sup>1</sup>	Following rain wind rising	77-81%	72%	72-77%	74%
27th—all day <sup>1</sup>	Bright	77-81%	71%	69%	60%

Several points may be noted from the preceding table with respect to the atmospheric humidity at the four locations:

1. Only on rainy days was the relative humidity in the sawmill, outside the sawmill, and at the top of the log-pile where *Fomes roseus* fruited as high as that in the log-pile where *Trametes subrosea* fruited.

<sup>1</sup> Several observations between 9 A.M. and 6 P.M.

2. The relative humidity in or near the sawmill and on the top of the log-pile was never higher than that down in the log-pile.

3. The relative humidity in the sawmill where *Fomes roseus* fruited was about the same as that upon the top of the log-pile where this fungus fruited also, and where *Trametes subrosea* did not fruit (and apparently could not fruit, as is shown below).

4. The relative humidity in the sawmill was 6 per cent to 10 per cent lower most of the time than that inside the log-pile or down in the shelter of the sweet fern where the *Trametes subrosea* fruited.

5. The relative humidity near the beam outside the sawmill with the *Fomes roseus* was drier at all times than the sawmill.

As to the fruiting of these two species at least, it would appear that *Fomes roseus* could tolerate somewhat drier atmospheric conditions than *Trametes subrosea*. Tests were made in August, 1922, to check these conclusions, by changing the places of the bolts bearing the two species respectively. Bolts on the top of the pile bearing the former species were placed down at the bottom, in the protection of the sweet fern, and bolts from down in the protection of the sweet fern bearing the latter species were placed on top in the open. The expected happened. The sporophores of *Trametes subrosea* (transferred from the bottom to the top) immediately dried up, became rigid instead of rubbery, revived during the next rain and then dried up to revive no more. No change was noted that year in the *Fomes roseus* placed down low among sweet fern.

When these bolts were examined the next fall (1923), *Trametes subrosea* fruit bodies had appeared alongside of the *Fomes roseus* on the bolts which had been transferred from the top to the bottom of the pile and the latter fungus seemed to be fruiting normally. Identification of these forms was made morphologically and culturally. On the bolts on the top of the pile, the dead *Trametes subrosea* fructifications of the previous year were still there, and also a few inches away on two bolts was a new fruit body one season old. These appeared to be *Fomes roseus*. One half of each was cut off for study and the other half of each left for further observation. Cultures made from each of these halves run at 30° C. proved the species to be *Fomes roseus*.

That same fall (1923), the bolts which had been removed from the top of the pile to the bottom in 1922 with the changes noted and upon which *Trametes subrosea* had now fruited in the moister conditions, were changed back to the top again. The other bolts brought from the bottom to the top which had developed *Fomes roseus* were left on the top. When all these were examined in the fall of 1924, it was found: that the halved fruit bodies of *Fomes roseus* had grown another layer of tubes, substantiating the culture test; that no *Trametes subrosea* had developed on these exposed bolts in two years alongside the ones which had been there previously and died, and that the bolts which in 1922 had had no *Trametes subrosea* on top of the pile, but had developed some of these fruit bodies the next year down at the bottom of the pile, now had only the dried fruit bodies of this species, while the original *Fomes roseus* was continuing growth.

This working theory that *Fomes roseus* was better adapted for living and fruiting upon dry hewn timbers than was *Trametes subrosea* and would be found more often in drier places was vindicated many times in the White Mountains through several summers.

This same difference in fruiting was noted also in wood block cultures in flasks (PLATE 34). *Fomes roseus* always fruited higher in the flask where it was drier. The arrow "A" at the *Fomes roseus* flask shows the level up to which fruit bodies grew in considerable abundance, the normal limit in flasks, although occasionally a few are formed higher than this. The lower arrow "C" near the "*Trametes carnea*" flask shows the normal upper limit of sporophore production by this fungus in flasks; the upper arrow "B" shows the absolute limit, which was approached only occasionally and then only in small numbers. In this particular flask, the sporophore showing at this level is the only one in the flask, and in fact was the only one that high in a dozen or more flasks of that series.

#### SUMMARY

The value of temperature responses of the mycelium as a means of differentiating cultures of wood-destroying fungi is elaborated. A few pairs of closely related fungi are used to illustrate the feasibility of using this reaction.

The temperature test is applied for the purpose of distinguishing *Fomes roseus* (Alb. & Schw.) Cooke and *Trametes subrosea* Weir, and also as contributory evidence of their specific difference.

*Trametes Feei* Fries, the only other polypore in this country with a rose-colored hymenium, was also included in these tests as a matter of interest.

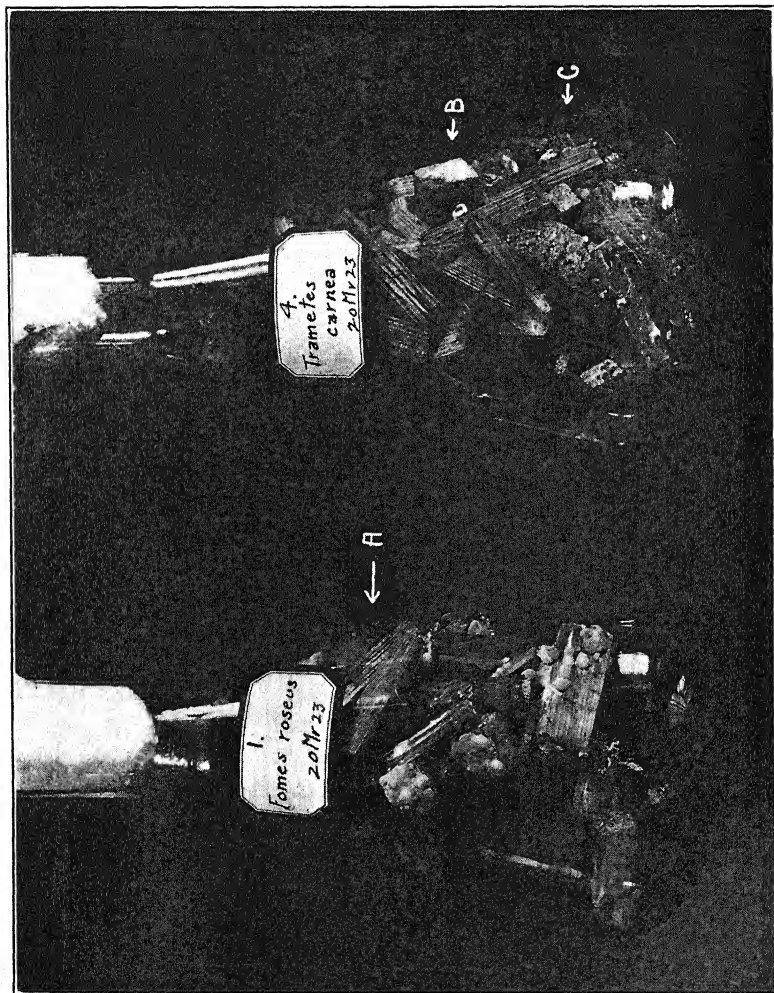
Data are adduced to show that *Fomes roseus* and *Trametes subrosea* are different also in their moisture requirements, or dryness tolerances, with regard to fruiting if not to growth. This was shown to be true not only by observation and experiment in the field, but also in wood block cultures in the laboratory.

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FOMES AND TRAMETES



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#### EXPLANATION OF PLATE 34

Sporophore production by *Fomes roseus* and *Trametes subrosea* (*T. carnea*) on wood blocks in flasks, in relation to relative humidity of the air.

It is shown that the fructification under flask conditions by *Trametes subrosea* is confined for the most part to the lower portion of the flask where it is moister (arrow "C"), with only occasional fruiting higher where it is somewhat drier (arrow "B"), and that *Fomes roseus* normally fruits in abundance as high in flasks as arrow "A," where it is considerably drier. Cultures 9 months old. The arrows refer to levels projected horizontally to the front of the flasks (middle of the flasks in the photograph).

## STUDIES ON SOME CALIFORNIA FUNGI

LEE BONAR

(WITH 2 TEXT FIGURES)

LASIOBOTRYS AFFINIS Hark.

References: Harkness, H. W., Bull. Cal. Acad. Sci. 1: 42, 1884. Thiessen, Ann. Mycol. 16: 175, 1918. von Höhnelt, F. Ber. Deut. Bot. Ges. 37: 103, 1919. Sydow, Ann. Mycol. 18: 181, 1920.

On *Lonicera hispidula* Dudl. var. *californica* Jepson.

Found commonly on the above named host in the Coast Range of western California. A specimen of the type collection from Mt. Tamalpais is deposited in the Herbarium of the Cal. Acad. of Sci.

Ellis listed this plant as synonymous with *Lasiobotrys Lonicerae* Kunze, but it was shown by Thiessen to be very different from *Lasiobotrys Lonicerae*. He examined some of the type material and gave a somewhat fuller description of the plant and some figures. A comparison of his figures with those for *L. Lonicerae* readily points out the striking differences between the two plants.

Von Höhnelt discussed at length the structure and taxonomic position of the genus *Lasiobotrys*, being of the opinion that it should be placed in the Dothideales. He was handling, for his American material, that distributed by Ellis as *Lasiobotrys Lonicerae* from *Symphoricarpus*. This has been shown by Sydow to be a true Dothideaceous fungus and named *Rhizogone Symphoricarpi*.

Von Höhnelt listed the imperfect stages of the genus, so far as any were known, under the genus *Kabalia* of Bubak. These had been referred to *Labrella* by Desm. and to *Leptothyrium* by Saccardo. These forms have been assumed to be the imperfect stages because they are found constantly associated with *Lasiobotrys* on *Lonicera* species.

I have collected *L. affinis* many times and at all seasons of the year in California and have not seen any evidence of such, or any

other forms of Imperfecti, associated with the infection on *Lonicera*.

The ascospores of *L. affinis* germinate rather slowly in water. Isolations of single spores have been made and the plant grown in the laboratory for a period of two years. The mycelium is brown to black, in the culture, and forms a scant aërial growth, over the medium which becomes black in appearance. Conidiophores soon form on the mycelium. They are erect dark hyphae and bear a conidium at the tip. The conidium is at first 1-celled and sub-hyaline. The conidiophore continues to grow from below the conidium so that it is pushed aside and then another conidium forms at the tip. This continues until three or four conidia are formed, in many cases, on a single conidiophore. The conidia remain attached and continue to grow, becoming several celled, and the walls covered with tubercular projections and brownish black. These spores when mature are globoid to ellipsoid, with rough wall, muriform, 12–15 microns in diameter and up to 25 microns in length (see Figure 1).

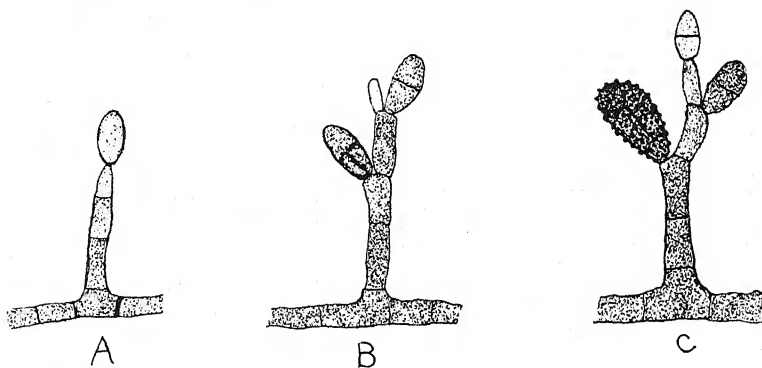


FIG. 1. *Lasiobotrys affinis* Hark. A–C showing stages in the development of the conidiophores and conidia

Long-continued cultivation of the fungus on a wide variety of media and under varying conditions has failed to give anything other than this hyphomycetous form.

I have not seen this conidial form on the host plant. Very clear evidence is here afforded that *Lasiobotrys affinis* Hark. is distinct from other species of this genus listed on *Lonicera* and the following amended description is offered.

*LASIOBOTRYS AFFINIS* Hark.

Spots scattered, amphigenous, subcuticular in origin, forming black spots which are rather elevated, slightly convex, and 2-10 mm. in diameter. These consist of a thin black hypostroma, from which rise flat-topped columns of sclerotial material. The perithecia nestle free between these sclerotial masses which are slightly higher than the perithecia. Perithecia globose, chestnut brown, 70-100 microns in diameter, few stiff hairs as outgrowths from the perithecial wall. Asci short-stipitate, nearly cylindric, 50-70  $\times$  12-15 microns. Spores ellipsoid to fusoid, very unequally 2-celled, light brown in color, and 14-17  $\times$  6-8 microns. No paraphyses.

*Dothidella castanicola* comb. nov.

(*Phyllosticta castanicola*) Ellis & Ev. Proc. Acad. Nat. Sci. Phil., 431, 1895.

(*Dothidella Castanopsidis*) Dearn. Mycologia 16: 155, 1924.

Type collection from Sisson, Cal., on *Castanopsis chrysophylla* (Hook.) DC. Perithecial stage described from collections in Oregon on the same host, by J. S. Boyce. Collections during the past few years have shown it to be rather common throughout the California Sierras on *Castanopsis sempervirens* Dudley. Fresno Co., 1921, Bonar; Mariposa Co., 1922, Kawagoe; Tuolumne Co., 1923, Mason; Eldorado Co., 1924, Parks and Fields. These collections have included both the pycnidial and the perithecial stages of the fungus.

Single spore cultures of the fungus were started from pycnidiospores and from ascospores and grown in parallel series. The cultures from the two sources were identical in behavior in culture, in so far as could be observed. Pycnidia were formed, rather sparingly, on a number of different kinds of substrata, in both of the series of cultures. The spores from these were like those from the pycnidia on the host tissue and show agreement between the two series, so that there can be no doubt that the two described forms from this host plant are stages of the same plant.

Continued cultivation, from numerous isolations, and on a wide variety of different types of media, failed to yield any sort of reproductive body other than the pycnidia.

**Leucostoma Sequoiae** sp. nov.

Stromata scattered, erumpent, 2-3 mm. in diameter. Imbedded in the bark with the ostiola showing as black points. Limits of the stromata formed by a distinct black line, while the inner tissue in which the perithecia are imbedded is cinereous and fibrous in texture. Perithecia 8-12 in a stroma, in a single plane,  $\frac{1}{4}$ - $\frac{1}{2}$  mm. in diameter, with a long, slender neck which penetrates a surface disk of somewhat firmer grayish material. Asci numerous, cylindric-clavate,  $45 \times 7$  microns. Ascospores allantoid, hyaline, not strongly curved,  $8-11 \times 2-3$  microns.

Imperfect stage *Cytospora*, scarce on the type material. Stromata similar to those of the perithecia except that they are composed of harder, more carbonized material, as is typical for the pycnidial stage of this genus. Pycnidial stromata plurilocular, or imperfectly so, conidiophores filiform, branched, interspersed with numerous sterile hyphae, which protrude into the cavity of the pycnidium. Spores allantoid, hyaline,  $4-6 \times 1.5-2$  microns. Spores may be extruded in yellowish masses when the twigs are moistened.

Single ascospore isolations were made of this material and it was grown in pure culture, on various types of media, for a period covering three years. Pycnidial stromata have been found in abundance in cultures but no perithecia have been formed.

On dead twigs of *Sequoia sempervirens*.

Collected Mill Valley, California, May 20, 1923.

**MELANOMMA SEMINIS** (Cooke & Hark.) Sacc.

Collected, Berkeley, Cal., on dead stems of *Baccharis pilularis* DC. and of *Urtica gracilis* Ait. var. *holosericea* Jepson.

The collection on *Urtica* adds a new host for this fungus.

Single ascospore isolations were made, and the fungus grows readily in artificial media of various kinds. After 7-10 days pycnidia are formed in the cultures and these, when mature, are found to belong to the form genus *Phoma*. Pycnidia globular to pyriform, papillate, 100-150 microns in diameter; spores abundant, ellipsoid, hyaline,  $4-6 \times 2-2.5$  microns.

**PHOMA THERMOPSIDICOLA** P. Henn.

On leaves and stems of *Thermopsis macrophylla* H. & A., Mt. Tamalpais, Calif.

Found causing large blackened areas on the leaves in late summer. The pycnidia are scattered over the infected areas and ap-

pear on both surfaces of the leaves. About the time that the leaves are shed the pycnidia appear in abundance on the dead and dying stalks and are to be found in abundance during the winter months. This appears as a new host and regional record for this fungus which was originally described from material from the Berlin Botanical Garden on *Thermopsis fabacea*, which is native to Kamtchatka.

*Phyllosticta sparsa* sp. nov.

Spots circular to subcircular, brown, becoming bleached in age, surrounded by a slightly elevated brownish-black line, tissue outside this line reddish brown. Spots equally distinct on both sides of the leaf, up 3-5 mm. in diameter. Pycnidia scattered, few, on the upper surface only, sub-epidermal, erumpent, globose, reaching a diameter of 150 microns, spores globular, contents conspicuously granular, 9-12 microns. Conidiophores simple, short, up to the diameter of the spore in length.

On leaves of *Vaccinium ovatum* Pursh., Mt. Tamalpais, Marin Co., Cal., Oct., 1925.

PHYLLOSTICTA INNUMERA Cooke & Hark.

Seaver, *Phyllostictales*, N. Am. Fl. 6: 71, lists this species as doubtful, as no host was given in the original note describing it in Greville. Cooke and Harkness, in their *Fungi of the Pacific Coast*, Bull. Cal. Acad. Sci. 1: pt. 1, p. 14, 1884, list this fungus as parasitic on the living leaves of *Fraxinus oregana*, from Mt. Tamalpais, in California, and the type specimen, so labeled, is deposited in the Cal. Acad. of Science.

This plant agrees in every way with the description given for *Phyllosticta viridis* Ellis & Kellerm., and the name of Cooke and Hark. should supercede the later one. I have found this fungus common on the leaves and fruits of *Fraxinus oregana* in the coastal region north of the Golden Gate, and it sometimes causes severe defoliation in late summer.

Harkness listed (Bull. Cal. Acad. Sci. 1: 160, 1885) *Phoma samararum* Desm. on the fruits of *Fraxinus oregana*. I have collected in the locality listed by Harkness and found the fruits of this tree commonly bearing the fruiting bodies of a fungus which are in every way identical with those of *Phyllosticta innumera*. This fruit infection is always associated with the leaf infection, so



that there is no doubt that the two forms are the same plant species. The fungus found on the fruits here is quite different from that described as *Phoma samararum* Desm. from Europe.

**Phyllosticta Lupini** sp. nov.

Spots irregular in size and shape, sometimes covering almost the entire leaflet. Upper surface little changed at first, becoming yellowish to brown, while the lower surface is black, due to the closely crowded pycnidia which are set in the leaf tissue. Effect is a slow killing of the tissue and the infected leaflet rolls inward, and in many cases a large percentage of the leaves of the plant is killed.

Pycnidia hypophyllous, globular, sub-epidermal, pushing up the epidermis so as to appear almost superficial at maturity, and very closely crowded together. Ostiole poroid. Pycnidia 100–150 microns in diameter. Conidia elliptical, hyaline,  $4-6 \times 2-3$  microns.

On leaves of *Lupinus succulentus* Dougl., Tiberon, Calif., March, 1923. H. E. Parks. On *Lupinus micranthus* Dougl., Humboldt Co., Calif., by Parks, 1923.

Common in San Francisco Bay region, March to July.

## STUDIES IN THE GENUS HARKNESSIA

Cooke, *Grevillea* 9: 81, 1881; 13: 111, 1884.

Winter, *Hedwigia* 22: 19, 1883.

von Höhnelt, Sitzb. Akad. Wiss., Abt. 1, 118: 1537, 1909.

The type species of this genus, *Harknessia Eucalypti* Cooke & Hark., on *Eucalyptus globulus*, was described from material collected in California. Cooke listed the genus as one of the Melanconiales. Winter placed it in the Sphaeropsidales and has been followed by numerous writers. Von Höhnelt made a study of material from European herbaria and came to the conclusion that it belonged in the Melanconiales. He listed all the numerous described forms from various parts of the world under two species—those occurring on *Eucalyptus* from various parts of the world as *Harknessia uromycoides* (Speg.) Cooke, and that on *Arctostaphylos* as *Harknessia Arctostaphyli* Cooke & Hark.

Since these two species were first collected within a few miles of each other and there seemed such very slight differences between them, I undertook a careful study of the two forms. I

have collected both these species in their respective type localities and have compared my material with the original Harkness collections in the Cal. Acad. Sci. Herbarium.

*HARKNESSIA UROMYCOIDES* (Speg.) Cooke.

Fruit body globoid, black, about 0.5 mm. in diameter. Sections through young ones show the upper layer to be a thin black membrane, which breaks away rather tardily, leaving a mat-like acervulus which appears as superficial, although it originated under the epidermis or periderm. Conidiophores, which remain attached to the spores when they are set free, 100–150 microns in length by 4 microns in diameter. Spores jet black when mature, with a hyaline papilla at the distal end,  $24\text{--}32 \times 12$  microns.

Found on the leaves, twigs, and fruits of *Eucalyptus* species from California in U. S., Spain, Argentina, and Tasmania.

*HARKNESSIA ARCTOSTAPHYLI* Cooke & Hark.

Fruiting bodies similar to those for the above described species. Conidiophores 20–40 microns in length, with a much thickened base. Spores jet black,  $18\text{--}24 \times 11\text{--}13$  microns, and lacking any papilla at the end.

Found on dead leaves of species of *Arctostaphylos*, Mt. Tamalpais and Berkeley, also on fallen leaves of *Arbutus Menziesii* Pursh. on the slopes of Mt. Tamalpais.

These two species have been grown in pure cultures from single spore isolations in parallel series and the morphological distinctions named above remain constant throughout the series. Fruit bodies are formed on a wide variety of media but most abundantly on those rich in sugars. *Harknessia Arctostaphyli* grows very sparingly and fruits rarely when grown on sterilized leaves or twigs of *Eucalyptus*, while *Harknessia uromycoides* fruits very abundantly on the same material. The latter species is more cosmopolitan as to its food requirements and has been widely distributed over the world with the distribution of the host species. *Harknessia Arctostaphyli* is more specific as to its food requirements and is known only from the type locality on the dead leaves of *Arctostaphylos* and *Arbutus*.

The development of the elongated conidiophores of *H. uromycoides* has been followed. The conidiophore is about the same length as the spore at the time that the spore attains approximately

its full size, although it is at that time sub-hyaline instead of black. As the spore wall becomes darker there is a very rapid elongation of the conidiophore until it reaches the lengths given for it. In a young fruit body the conidiophores will be found to be of lengths varying 25–150 microns, all bearing spores that are approximately the same size, while in the older fruit body, from which the outer wall will be broken away, the conidiophores will be found to be uniformly of the greater length, and they break away at the base, remaining attached to the spores.

**Disaeta** gen. nov.

Melanconiaceae-phaeophragmiae.

Acervuli intra-epidermal or subcuticular, erumpent, discoid, black; conidia elongate, fusoid, colored, with the end cells hyaline; bearing one hyaline bristle at each end of the conidium.

Like *Hyaloceros* Dur. (*Monochaeta* Sacc.) except that there is a bristle at each end of the conidium.

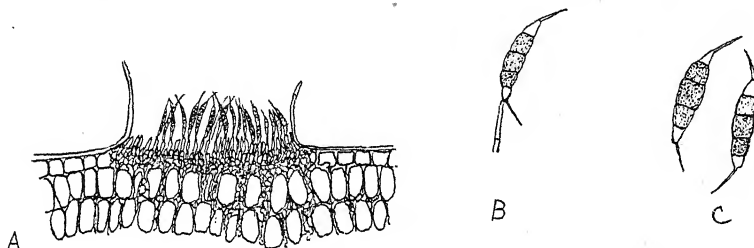


FIG. 2. *Disaeta Arbuti*. A. Section through an acervulus; B. Conidiophore with conidium attached; C. Typical conidia

Differs from *Pestalozzia* Sacc. in having the central cells colored, which places it in the Phaeophragmiae.

Type species, ***Disaeta Arbuti***.

Spots irregular in outline, often becoming several centimeters in diameter and involving the major portion of the leaf, dark brown with a purplish black border, which is more evident above. Spots tend to break up and fall out in angular pieces. Acervuli epiphyllous, scattered, often concentrically arranged, .25–.5 mm. in diameter or becoming confluent; intra-epidermal, erumpent by the breaking of the cuticle. Conidia abundant, fusoid, slightly curved, typically 5-celled, the end cells hyaline, the central ones sub-opaque. Each of the terminal cells set with one bristle-like hair, averaging 7 microns in length. Conidia  $18-26 \times 4.5-7$

microns; conidiophores simple, one-half the length of the conidia. See figure 2.

Parasitic on the leaves of *Arbutus Menziesii* Pursh., Mt. Tamalpais, Marin Co., Cal., and Oakland, Cal., Jan., 1923.

Single spore isolations were made and cultural reactions on a variety of types of food material determined. Growth is very slow on synthetic media containing various sugars as the course of carbohydrate food, and likewise on starchy food, as afforded by cornmeal agar. Growth is very vigorous on oatmeal agar and on sterilized green beans, and abundant spore formation occurred on these latter media after 1-2 months.

*PESTALOZZIA CASTAGNEI* Desm.

On living leaves of *Lithocarpus densiflorus* (H. & A.) Rehd. (*Pasania*) (*Quercus*), Muir Woods, Calif., Jan. 13, 1923.

Not heretofore reported on this host or from this region. Found commonly in the outer Coast Range in central California, where the host plant is found. Acervuli abundant on the upper surface of the leaves as black specks .5-1 mm. in diameter. Infection usually starts at the tip of the leaf and gradually spreads to kill the larger portion of the leaf.

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## LOPHODERMIIUM ABIETIS ON PSEUDOTSUGA TAXIFOLIA

J. S. BOYCE

For the past 50 years species of the genera *Hypoderma*, *Hypodermella*, and *Lophodermium* and the damage they cause to young conifers have figured prominently in European literature, while within the past few years there has been an increasing interest among mycologists and forest pathologists in North America in the same fungi. New species have been described, new hosts recorded, and knowledge of distribution extended. Not only do these fungi comprise an attractive group for study but they are becoming increasingly important from an economic standpoint as the United States and Canada more and more turn their attention to timber growing. Commonly endemic on young conifers, they often become epidemic and considerable loss results.

Owing to the extremely important position that Douglas fir (*Pseudotsuga taxifolia* (Lam.) Br.) occupies and will continue to occupy in the forests of the Pacific Northwest, any parasite attacking this species demands attention. *Lophodermium Abietis* Rostrup, which attacks and kills the needles of young firs and spruces, has been reported on Douglas fir in Denmark by Rostrup (3, p. 527). This is the only record of this fungus on Douglas fir known to the writer, later records always referring to the observation by Rostrup. The writer has never found the fungus on Douglas fir. Throughout the Western United States *L. Abietis* is not uncommon on Pacific silver fir (*Abies amabilis* (Loud.) Forbes), white fir (*A. concolor* Lindl. & Gord.), lowland white fir (*A. grandis* Lindl.), alpine fir (*A. lasiocarpa* (Hook.) Nutt.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) where the species are mixed with Douglas fir, but the latter is not infected. In Great Britain in 1925 the fungus was found on fallen needles of Sitka spruce and Norway spruce (*Picea excelsa* Link.), but young Douglas firs in the immediate vicinity were not affected. Again, on the Island of Bronholm in Denmark no trace of the fungus was

seen in the Douglas fir plantations in the State Forest of Almindingen when they were visited in September, 1925.

The only American record the writer has been able to trace, transmitted through the courtesy of John Dearness, was a collection made by V. Simmons at Coldspring, Albany County, Wyoming, June 23, 1917. The host was labelled *Pseudotsuga taxifolia*, but a critical study of the needles proved it to be alpine fir.

Lind (2, p. 147) has reported the fungus on Douglas fir in Denmark, but an examination of the collection in Rostrup's herbarium at Copenhagen in September, 1925, on which this report was based showed it to be a mixture of Douglas fir and spruce needles, with the apothecia of the fungus occurring on the spruce needles only. The needles appeared to be those of Norway spruce. No other collection of *L. Abietis* on Douglas fir was found in Rostrup's herbarium. Recently (1, p. 486) it has been pointed out that attacks of consequence on Douglas fir have never been observed in Denmark and it is recommended that this species, because of its great resistance to the fungus, be given preference over Norway spruce for planting in the coast region.

It may be concluded that it is highly doubtful if *Lophodermium Abietis* attacks Douglas fir and past records have been based on an error in host determination.

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#### LITERATURE CITED

1. Fabricius, O. 1926. Douglas- og Sitkagran. In Dansk Skovforen. Tidsskr. no. 3, pp. 405-541, figs. 1-24, August.
2. Lind, J. 1913. Danish fungi as represented in the herbarium of E. Rostrup. 650 pp., 42 figs., 9 pls., February. Copenhagen.
3. Rostrup, E. 1902. Plantepatologi. 640 pp., 259 figs., March. Copenhagen.

## NOTES AND BRIEF ARTICLES

The University of Michigan Herbarium has received from Dr. Howard A. Kelly of Baltimore, as a gift, his magnificent Mycological library and his collections of photographs and paintings of mycological subjects, as well as a set of higher fungi and lichens along with a fine exhibition group of the higher fungi. It was accepted by the University on April 24. It is to be called the "L. C. C. Krieger Mycological Library and Collection" in commemoration of this artist-mycologist. About 350 paintings of mushrooms by Krieger are included. The library has an index of about 10,000 numbers. Along with the Phanerogamic and Cryptogamic Herbarium now at the University, and under the Directorship of C. H. Kauffman, the Kelly gift is installed in the New Museum Building just completed at Ann Arbor. It is expected to be available soon to the scientific public.

\* C. H. KAUFFMAN.

### THE C. G. LLOYD MYCOLOGICAL COLLECTION

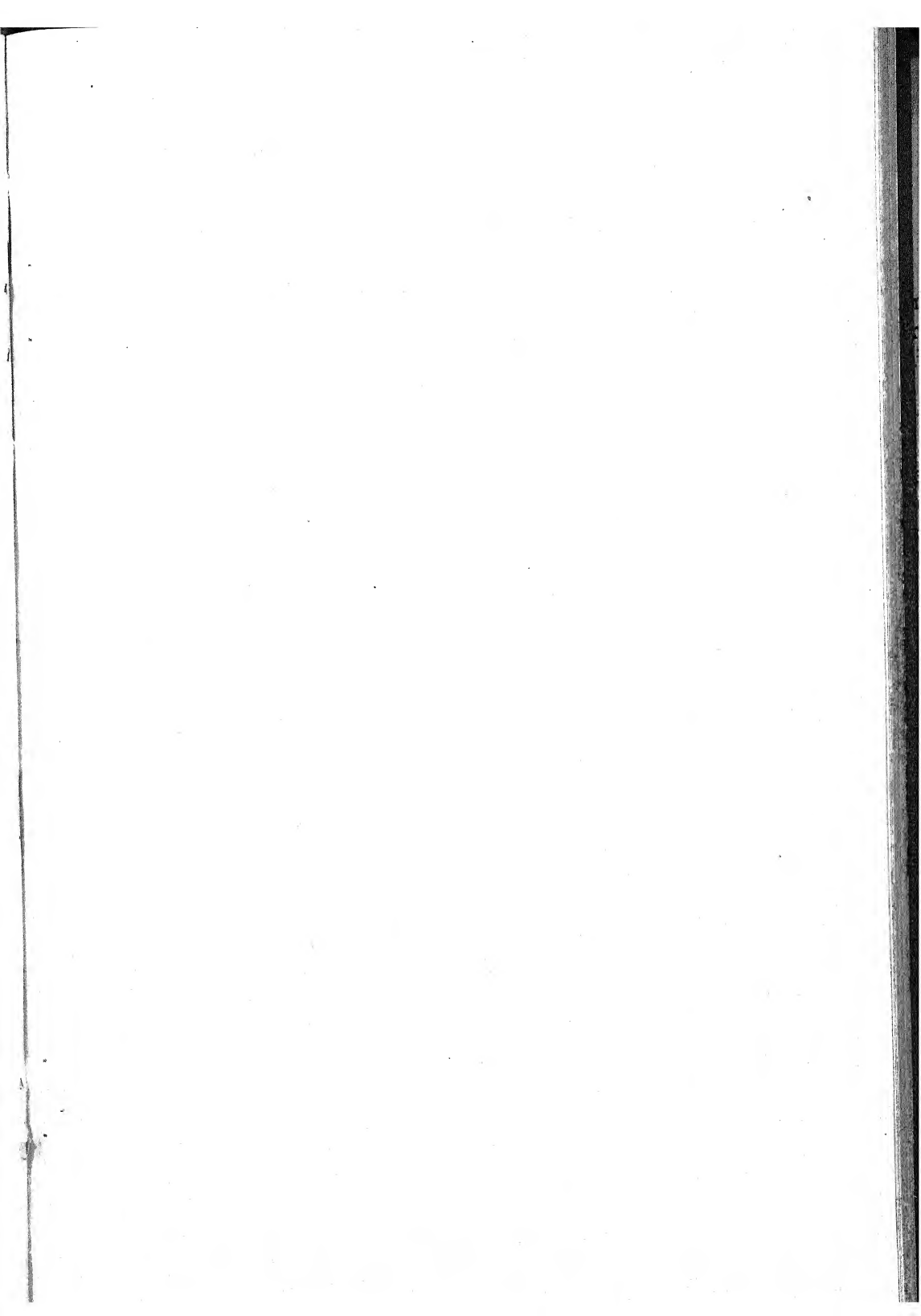
During Mr. Lloyd's last visit to Washington he expressed a desire to have his Herbarium brought here, and tentative plans were made. Later, on account of ill health, he decided that he would be unable to carry out the plan. Since Mr. Lloyd's death, a coöperative arrangement has been made between the Smithsonian Institution and the Department of Agriculture to take charge of the collection, and the trustees of Mr. Lloyd's estate have transferred the collection to Washington, where it will be installed in fireproof cases in the Bureau of Plant Industry, as a separate unit in connection with the present Mycological Collections.

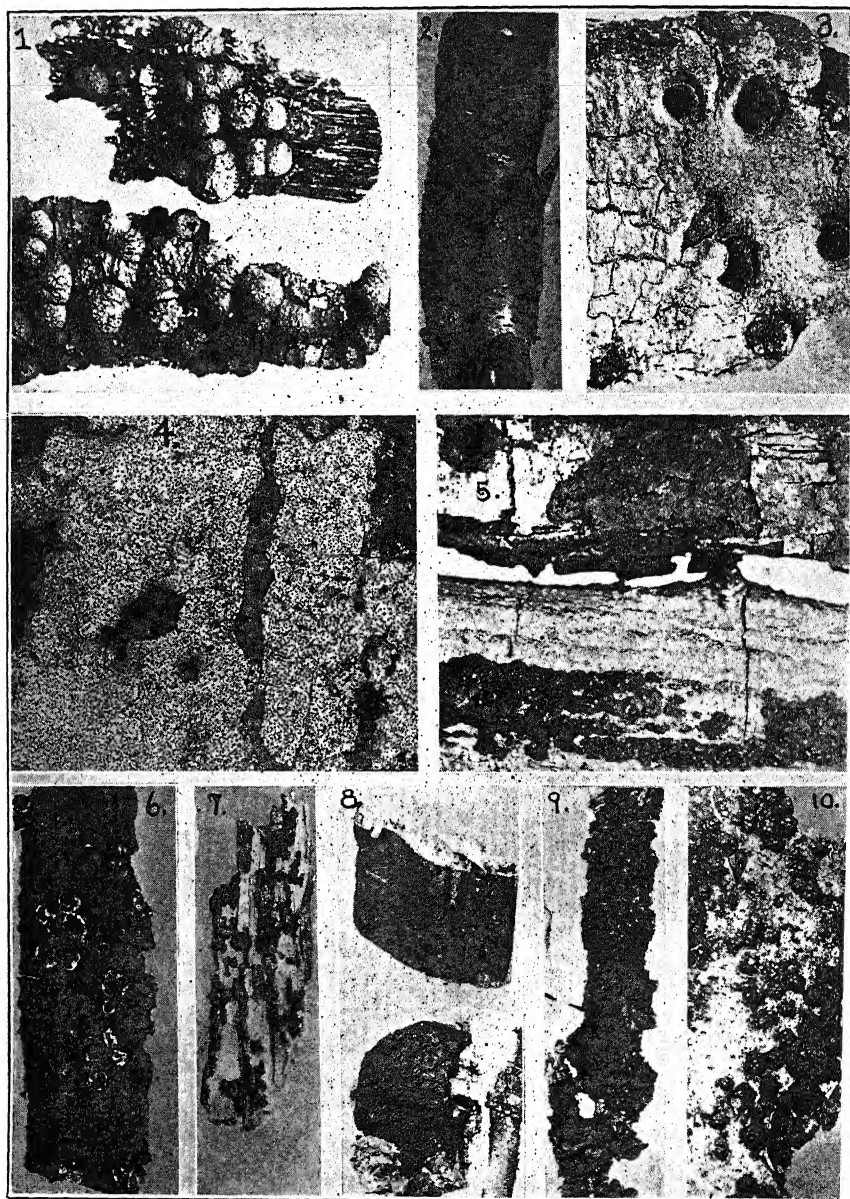
This collection represents the life work of Mr. Lloyd, and contains according to his estimate about 100,000 specimens, consisting chiefly of the larger Hymenomycetes and the larger Ascomycetes. Many type specimens are included not only of species described by Mr. Lloyd, but also those of other mycologists; also nearly 10,000 negatives illustrating fungi, hundreds of

photographic prints and half-tones of all illustrations published by Mr. Lloyd, and his voluminous correspondence with most of the mycologists of the world. All of the material has been safely received in Washington, and will be catalogued, arranged and made available for study as soon as practicable.

The collection is particularly rich in material from all parts of the Tropics and little-explored regions of South America, Africa, Australia and other countries. C. L. SHEAR.







XYLARIACEAE

# MYCOLOGIA

VOL. XX NOVEMBER-DECEMBER, 1928 No. 6

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## BIOLOGIC STUDIES IN THE SPHAERIALES—II <sup>1</sup>

JULIAN H. MILLER

(WITH PLATES 35-38)

In Part I (18) of this investigation the writer has shown that many fungi now placed in the Sphaeriales have their asci borne in unilocular stromata. This type of development is found in the Dothideales. When these fungi are taken out, the order Sphaeriales will be characterized by the possession of a definite type of perithecium. This fructification will be defined as a globose to flask-shaped conceptacle, which arises from an archicarp, and opens by an ostium that is formed by the upward growth of the wall. Inside of the mature perithecium are to be found asci and paraphyses lining the base and sides and paraphyses lining the ostium. Stroma is always found on the outside of the perithecial wall. The presence of this stroma and the ostiolar characters will serve to distinguish this order from the Laboulbeniales and the Erysiphaceae.

The question arises as to how this order should be divided into families which will bring together related forms. The arrangement of the species in an ideal system of classification would

<sup>1</sup> Also presented to the Faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

This investigation was accomplished under the direction of Dr. H. M. Fitzpatrick to whom the writer wishes to express his appreciation for his suggestions and continued helpful supervision of the problem. The writer is also indebted to Dr. L. M. Massey for making available the herbarium and the facilities of the laboratory, and to Prof. H. H. Whetzel for much inspiration and encouragement in the prosecution of this investigation.

[MYCOLOGIA for September-October (20: 251-304) was issued  
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certainly be based on natural relationships. The members of such a system would constitute an evolutionary series, beginning with the most primitive, and ending with the most highly specialized. In order to determine whether or not the species one groups together are phylogenetically connected, their life histories should be studied. Such investigations should serve as the basis for all taxonomic work.

Relationships in the Sphaeriales should be established on such important characters as the manner of development of the perithecium, types of asci and ascospores, and paraphyses and conidial characters. Such characters as the amount of stroma present, and the depth to which the perithecium or stroma is sunken in the substratum, are apparently most easily influenced by the environment, and so should not be used for major separations.

The object of this investigation is to show that the species with continuous dark-colored spores constitute a closely related group, all the members of which should be in the Xylariaceae, and further to determine the generic separations in that family. The common forms in this series are now placed in the genera *Sordaria*, *Melanospora*, *Rosellinia*, *Anthostomella*, *Anthostoma*, and the genera of the present family Xylariaceae. The writer will discuss these genera and will give the results of developmental studies in *Rosellinia*, *Hypoxylon*, *Ustulina*, *Nummularia*, and *Daldinia*.

Saccardo (24) placed these fungi in one group, the Phaeosporae, and based this entirely on the characters of the ascospores. In Lindau's (16) system of classification the family separations in the Sphaeriales are based on stromal characters, depth to which the perithecium or stroma is sunken in the substratum, the presence or absence of paraphyses, and the type of conidial fructification.

*Sordaria* in the Sordariaceae, *Rosellinia* in the Sphaeriaceae, and *Melanospora* in the Hypocreales, all have the same general perithecial characters, yet Lindau places them in widely separated groups along with other fungi which have entirely different types of asci, ascospores and paraphyses. Also *Anthostomella* is placed in the Clypeosphaeriaceae, and *Anthostoma* in the Valsaceae, and

the single difference between those genera is that the perithecia are single in *Anthostomella* and aggregated in *Anthostoma*. The members of the Xylariaceae, as understood by Lindau, represent the well-developed stromatic forms in the dark-spored series and constitute a related group.

#### HYPOXYLON Fries

##### Historical Sketch of the Genus

This generic name was created in 1791 by Bulliard (2), and the genus was placed in his first order of fungi which he separates on the basis of the internal origin of the spores. In this order are found what are now considered Myxomycetes, Fungi Imperfecti and Gasteromycetes as well as some Sphaeriales. Bulliard published sixteen species under the name of *Hypoxylon*, eleven of which are not *Hypoxylon* species in the present sense. According to the American Code of Nomenclature the first species described should be the type of the genus, and as this is *H. sphinctericum* Bull., a form which is not a *Hypoxylon*, this would change the generic concept that has been generally accepted. The International Code would not take this work as the beginning point, but would start with the "Systema Mycologicum" of Fries (7).

Persoon in 1801 (22: 8), in his genus *Sphaeria*, sec. II, Periphericae, published what are now considered as *Hypoxylon*, numbers 11-19 and 30 and 32-35. The number 11 here is *Sphaeria concentrica* (*Daldinia concentrica* (Bolt.) Ces. & De-Not.). The other numbers are *Hypoxylon* and *Nummularia*. Between numbers 19 and 30 are placed chiefly *Hypocrea* species.

In 1822 Schweinitz (25: 28-48) continued Persoon's conception of the genus *Sphaeria*. In his second subgenus, Periphericae, numbers 12-20 are *Hypoxylon*; in his third subgenus, Compressae, numbers 27, 29, 36, 38, 39 and 42-46 are *Hypoxylon*; in his subgenus Monostichae 81, 82 and 87 are *Hypoxylon*; and in his seventh subgenus, Caespitosae, number 122 is a *Hypoxylon*. The intervening ones in these groups are now considered as *Hypocrea*, *Diatrype*, and other genera of the Sphaeriales. The first published, number 12, is *Sphaeria concentrica*.

Fries (7: 319-359), in "Systema Mycologicum" in 1823, also

continued the genus *Sphaeria*. Here species of *Hypoxylon* are mixed with other fungi. In tribe III, Pulvinatae, the first species is *Sphaeria globosa*, now *Xylaria globosa*, and the second is *Sphaeria concentrica*, the other six being *Hypoxylon*. In tribe IV, Connatae, ser. I, are to be found only species of *Hypocrea*, while in ser. II there are 13 species of *Hypoxylon*. Tribe V, Glebosae, contains 8 species of *Hypoxylon*. Tribe VI, Lignosae, contains 5 species of *Hypoxylon* mixed with species of *Diatrype*.

In 1849 Fries (8: 383) in his "Summa Vegetabilium Scandinaviae" recreated the genus *Hypoxylon* for the first time since the work of Bulliard from tribes of his former genus *Sphaeria*. He gives Bulliard credit for this generic name. Here the genus is divided into Glebosae, Pulvinatae, Effusae, and Connatae. In the first subgenus he places first *H. ustulatum* Bull. (*Ustulina vulgaris* Tull.), following this with species now considered *Nummularia*. In the Pulvinatae are *H. concentricum* and pulvinate species of *Hypoxylon*; and in the last groups, Effusae and Connatae, are found effused forms of *Hypoxylon*.

According to the International Code all members of the Sphaeriales start with "Systema Mycologicum" of Fries, but as he contained these fungi within the larger group, *Sphaeria*, the genus did not have its beginning in that work. Bulliard cannot be given credit for this, because he was previous to Fries, and also our present concept of the genus *Hypoxylon* could not possibly evolve from his generic description nor from the species he described. Therefore, Fries in 1849 in "Summa Vegetabilium Scandinaviae" creates the genus *Hypoxylon*.

What should be considered the type of the genus? Neither Fries nor Bulliard designated a type species. The fact that Fries gave Bulliard credit for the genus and then transferred five of the Bulliard species apparently indicates that he considered these species as typical of the genus. These species are 1. *H. ustulatum*, 7. *H. nummularium*, 11. *H. coccineum*, 13. *H. granulosum* (*H. multifforme* Fries) and 16. *H. glomeratum* (*H. fuscum* Fries). If one agrees with Tulasne (30) in raising *H. ustulatum* and *H. nummularium* to generic rank, then *H. coccineum* would probably be considered the type of the genus. Shear (27: 84) considers this species the type. The writer does

not agree with the change made by Tulasne and thinks it sufficient to designate the five species transferred from Bulliard's genus as typical of *Hypoxylon*.

The following description of the genus is given in order to more clearly define its limits and to incorporate data brought out in this investigation.

#### HYPOXYLON Fries, *emend.*

Stroma globose to pulvinate to effused, erumpent, fleshy when young, leathery, woody or carbonaceous when mature. Perithecia several to many in stromata; paraphyses numerous, thread-like; asci cylindrical, arranged on sides and bottom of perithecia; ascospores brown to black, with an elongate hyaline depression, and uniseriate in the ascus.

Conidial layer formed first on an exposed ectostroma, later developing on old stromata in favorable weather. Conidiophores branched, hyphomycetous, hyaline to greenish-brown; conidia minute, borne apically, one to many, becoming lateral by the sympodial growth of the hypha.

#### Genera Split Off from the Genus Hypoxylon

1. *Ustulina* Tul. Tulasne (30: 23) in 1863 established this genus on *Hypoxylon ustulatum* Fries (1849), which was *Sphaeria deusta* in Fries's "Systema Mycologicum" (1823). This should have been *Ustulina deusta* (Fries), instead of *Ustulina vulgaris* Tul.

The separation is based on the character of the conidial stroma. Tulasne understood this to be more thick, fleshy, and superficial than in the other *Hypoxylon* species. In this respect Nitschke (19) agrees with him. Nitschke (19: 3), under "conspectus generum, b. stromate hypoxyleo," separates these genera as follows: "*Ustulina*, . . . stroma repando-pulvinatum. *Hypoxylon* . . . stroma subglobosum v effusum." The writer does not consider this a separation as many species of *Hypoxylon* are "repando-pulvinatum." Nitschke (19: 21) says this single species of this genus was correctly separated from *Hypoxylon*, since it agrees much more with *Xylaria* in its unmistakably peculiar habit in the consistency of the conidial-forming hymenium.

2. *Nummularia* Tul. In the above work Tulasne (30: 42)

created this genus on *Hypoxylon nummularium* Fries, *Sphaeria discreta* Schw., and a new form, *Nummularia dryophila* Tul. This separation is based on the supposed discovery of a conidial layer being borne under the stroma. He says at maturity it becomes erumpent and then produces the same appearance as in other species of *Hypoxylon*. Tulasne (30: Tab. V, figs. 11-19) illustrates the development of *N. Bulliardii* Tul. The drawing shows short conidiophores, bearing globose conidia, under a definite pseudo-parenchymatous stroma. Nitschke (19: 3) separates this genus as follows: "II. Conidia sub stromatis strato supremo nato, deinde libera," following Tulasne. In regard to the development (19: 56) he says he regrets that our knowledge of the conidial apparatus is limited to the discoveries made by Tulasne with *N. Bulliardii* and *N. discreta*.

3. *Daldinia* De-Not. *Sphaeria concentrica* Fries was the basis for this genus. De-Notaris (20) in 1863 observed that the concentric zonation of the stroma was more pronounced in this species than in the other species of *Hypoxylon*, and therefore erected the genus *Daldinia*. Neither Nitschke (19), Fries (8), nor Tulasne (30) had recognized this separation.

4. *Camarops* Karsten. The type of this genus is *C. hypoxylodes* Karst., described by him (15: 53) in 1873. According to von Höhnelt (14: 1218), this is the same as *Nummularia lutea* (Alb. & Schw.) Nits., or *Sphaeria lutea* Fries in "Systema Mycologicum," or *Hypoxylon luteum* Fries in "Summ. Veg. Scand." Also he says *Bolinia tubulina* (Alb. & Schw.), used as a subgenus under *Hypoxylon* by Nitschke (19), is in the same category. *Solenoplea microspora* Starback, according to von Höhnelt, also belongs here. The name *Bolinia* was first raised to generic rank in 1882 by Saccardo (24), while the name *Camarops* was created by Karsten in 1873. *Solenoplea* was described by Starback in 1902. Theissen (29) recognized that these three genera were identical.

This group is distinct from *Hypoxylon* on perithecial characters. *Camarops hypoxylodes* contains very long cylindrical perithecia which are closely appressed in a black stroma.

#### Development in the Genus *Hypoxylon*

The manner in which the stroma and perithecium arise in *Hypoxylon Howeianum* Peck has already been described by the



writer (18). *H. coccineum* Bull., which resembles the former closely, was studied by Lupo (17). The latter found three types of hyphae in the stroma from the time of its emergence from the substratum: "those that form the major part of the stroma, those that form the perithecium and Woronin hyphae, and those that form the superficial layers and probably conidiophores." (PLATE 36, FIG. 2.) The first type of hyphae comprise the entostroma, the second type the ones that arise from the initial coil or archicarp, and the third make up the ectostroma which is pushed up by the developing entostroma. Lupo says further, "The formation of the perithecium is initiated by the massing of the hyphae into a circular knot, within the center of which 'Woronin hyphae' differentiate. The ascogonia develop from the cells of the 'Woronin hyphae' by rounding out, partially separating from each other, and increasing in size. The ascogonia do not drop to the bottom of the perithecium in the older stages, but come to lie comparatively close to the bottom by an expansion of the perithecial wall toward the periphery of the stroma."

Fuisting (9: 305) found four stromatic types of *Hypoxylon* represented by *H. cohaerens*, *H. coccineum*, *H. rubiginosum*, and *H. udum*. In the first two the entostroma is well developed, forming an almost globose stroma. In *H. rubiginosum* the perithecia are seated in an entostroma, which is very thin, so that their bases almost touch the wood. *H. udum* first appears as a thin hyaline ectostroma on the surface of the wood, and without special differentiation is covered with a felt-like conidial growth, and after the development of the perithecia this ectostroma is seen as a firm black covering to the perithecia. There is practically no entostroma. The perithecia are seated directly in the wood. He says a striking similarity governs in the conidial formation in this genus.

Later (9: 307) he says one perithecial development serves for the family. In *H. cohaerens* the perithecial initial appears as a spherical ball, which is early differentiated into conceptacle and hymenial tissue, whose "Woronin hyphae" lie embedded at the base of the first formed knot, a thin thread-like, irregular weft, firmly coiled. Paraphyses form from all the inner wall layer accompanied by delicate periphyses. The black tissue

over the stroma plays the rôle of a second covering to the perithecia. After a while the formerly spherical ball is changed by unequal growth into an upright, ellipsoid form, whose summit is perforated by converging tissue and becomes the ostiolum. He says the periphyses grow out from the basal cells resting on wall cells. Also the papilla grows up through the outer stroma.

The species of *Hypoxylon* studied by the writer can be placed in four distinct groups: (1st) Species with the mature stroma of woody texture, with red to purple ectostroma and dark-colored entostroma, and umbilicate ostiola—*H. Howeianum*, *H. coccineum*, *H. fuscum*, and *H. rubiginosum*. (2d) Species with carbonaceous stroma and an annular depression around the ostiolar papilla—*H. Malleolus*, *H. marginatum*, *H. annulatum*, *H. effusum*, *H. Michelianum*, *H. serpens*, and *H. illitum*. (3d) Species with very little to no entostroma and perithecia sunken in the substratum—*H. confluens*, *H. semiimmersum*, *H. udum*, *H. gregale*, and *H. Morsei*. (4th) Species with constantly effused, carbonaceous stroma (most of these are now in the genus *Nummularia*)—*N. Bulliardii*, *N. clypeus*, *N. punctulata*, *N. atropunctatum*, and *N. discreta*.

#### 1. Stroma Woody, with Red to Purple Ectostroma and Dark-colored Entostroma, and Umbilicate Ostiola

##### HYPOXYLON HOWEIANUM Peck

The stroma is always globose to hemispherical and symmetrical, except when compressed by adjacent ones, and is never effused or pulvinate. Most stromata are 3–12 mm. in diameter and 3–8 mm. thick.

The color of the ectostroma is bright brick-red, and in later stages is very similar to that of *H. coccineum* Bull. In very old specimens this darkens to almost black. The monostichous perithecia are borne in the periphery of the stroma. The ostiolar papillae do not project out beyond the ectostroma, but in developing raise the ectostroma slightly over each perithecium. The opening is umbilicate. None of the body of the perithecium is erumpent through the outer stromal layer. The asci are about 80–100  $\mu$  in length, the spore part being from 50–60  $\mu$  long. The ascospores are from  $3-3\frac{1}{2} \times 6-9 \mu$ .

This species is most common on species of *Quercus*, particularly *Q. alba*, *Q. stellata*, and *Q. montana*, although it has been found on many other hosts.

Shear (27: 84) says *H. Howeianum* Peck is equal to *H. coccineum* Bull. The writer has examined Peck's type of *H. Howeianum*, and finds the asci are 80–100  $\mu$  in length, and the spores are  $3-3\frac{1}{2} \times 6-9 \mu$ . This specimen is on *Pyrus Malus*. *H. coccineum* has asci 120–150  $\mu$  long and spores  $12-14 \times 5-7 \mu$ . This is true in Exsicc. Moug. et Nestl., Stirp. Vog.-Rhen. no. 273, which is cited by Nitschke (19: 29) and also by Fries (7: 332). This is on *Fagus*. In the United States *H. coccineum* occurs on *Fagus* and the writer has never found it on another host. *H. Howeianum* occurs on several hosts, and apparently has not been found on *Fagus*. Shear further says *Sphaeria enteromela* Schw., in Michener's herbarium, is a form of *H. coccineum*, and that the specimen consists of two pieces, one on *Fagus* and one on *Castanea*. The specimen on *Fagus* is immature, but the one on *Castanea* contains spores of typical *H. coccineum*, which according to him are  $10 \times 3 \mu$ . The confusion here is due to the two species having been mixed in this packet, and the one on *Castanea* is *H. Howeianum*, and the one on *Fagus* is probably *H. coccineum*. The writer has seen many specimens of *H. Howeianum* on oak in various herbaria, all of which were labelled *H. coccineum*. The two are apt to be confused in certain stages unless one examines the spores.

In February 1926, the writer inoculated twigs and wood of *Quercus alba* with ascospores, and left them outdoors all of the following spring and summer. The next fall stromata of various degrees of maturity were found in abundance. Also conidia were plentiful during rainy weather. Free-hand sections, as well as paraffin, were made from time to time and the development studied.

The ascospore germinates on the bark and the germ tube penetrates the periderm, ramifying between that tissue and the cortex. From this very fine hyphal threads penetrate the cortex and xylem, and their limits become circumscribed by a black line. Next, this plexus of hyphae within the bark swells enormously, rupturing that tissue. The cells in this layer by coalescing form

a definite stroma. At this period these cells are angular, pseudo-parenchymatous, and are bright orange in color. This layer is homologous with the ectostroma of *Diatrype stigma* (Hoffm.) Fries as described by Wehmeyer (31: 593). This ectostroma forms the outer coat to the developing entostroma.

The entostroma begins as a delicate pseudoprosenchymatous tissue directly under the ectostroma. At this time it is composed of fine parallel hyphae, about 3-4  $\mu$  in diameter. They appear steel-gray in color. These hyphae form a palisade layer with the growing point just under the ectostroma. Even at this stage there is a sharp line dividing these tissues. As growth proceeds here the bark is further ruptured and the young stroma appears, about 1-2 mm. in diameter.

Just as soon as the bark is ruptured and the air is let in, conidiophores appear on the ectostroma. The conidia are minute, about 4-6  $\mu$  in diameter, varying from globose to oblong, and are hyaline. They are borne in clusters of one to several on ends of branches. The cluster, or single spore, is forced to occupy a lateral position by a branch arising directly under it and growing upward. This development is common to the other *Hypoxylon* species. The ability to produce these conidia seems to be retained by the ectostroma throughout its life period. Mature stromata will become covered with this gray conidial layer in warm rainy periods during the winter. Plate 36, fig. 7, shows the manner of conidiophore branching.

Perithecial initials arise in the upper part of the entostroma just under the ectostroma. They are seen at an early stage and are carried upward with the growing palisade layer. At first a hyphal knot is noticed in this position. This grows in a peripheral manner and all the cells which ultimately compose the perithecium come from this knot. When this becomes about 30  $\mu$  in diameter, coiled deeply staining hyphae of large diameter appear in the center. These are "Woronin hyphae," and have been seen in all *Hypoxylon* species examined. They become closely septate and the segments give rise to ascogenous hyphae, paraphyses, and periphyses.

The perithecial centrum as exhibited by *H. Howeianum* is typical for this genus. (PLATE 36, FIG. 2.) The asci are located

at the base and sides of the perithecium, oriented toward the ostium. The ascospores are light brown, and lie uniseriately in the ascus. The wall of the latter is thin, hyaline, and not thickened at the tip. The asci are cylindrical and long stalked. The ascogenous hyphae give rise to from 8-15 asci. (PLATE 36, FIG. 6.) All do not mature at the same time. The paraphyses are very delicate and branched and at an early stage completely fill the centrum. Delicate periphyses line the ostium.

The texture of the stroma is constant even with old age. It is never brittle-carbonaceous as in *H. marginatum* or *N. Clypeus*. When young it is fleshy and in age it becomes leathery to woody. Other constant characters are the dimensions of the ascospores and asci and the bright brick-red color of the ectostroma.

#### HYPOXYLON RUBIGINOSUM Fries

The stroma in this species seems to be the most variable of all the *Hypoxylon* species. It is entirely plastic under different environmental conditions and this has led to much confusion. Many species have been created on these environmental forms. The writer has found no host specialization.

In February 1926, the writer inoculated species of *Fraxinus*, *Salix*, *Acer*, *Quercus*, *Castanea*, and *Viburnum* with ascospores. The inoculations were made on thick bark, very thin bark, completely decomposed bark, and on decorticated wood. In this manner many variations of form and color were gotten. Photographs taken from the resulting stromata are shown on Plate 35, figs. 5, 8, 9. The first is the purple-red form which has been called *H. fuscopurpureum* (Schw.) Berk. The second represents what has been considered as typical *H. rubiginosum*, and the third is typical of what is called *H. perforatum* (Schw.) Sacc.

The stroma is pulvinate or effused, indefinite in extent. On decorticated wood it is continuous for several inches, and often perfectly smooth with only a slight raise over each perithecium. On thick bark it is pulvinate and often resembles *H. fuscum*. (PLATE 35, FIG. 2.) The irregular pulvinate form seen in fig. 9 is on thin, very rotten bark. The perithecia often stand almost fully exposed on the stroma. Sometimes they are aggregated

and often they are entirely separate. In old age the ectostroma flakes off, exposing from one half to two thirds of the perithecial walls.

The young stroma is fleshy-leathery, at maturity woody, and in very old specimens crumbling. It is never carbonaceous and so can be sectioned.

The color of the stroma when young is bright brick-red to purplish-red to shades of reddish-brown, and it is extremely variable in these colors. The writer has found the color to be entirely dependent on the moisture content. The brighter colors develop under moist conditions and the drab-brown colors under dry conditions. In old age the stroma becomes black.

The interior of the ectostroma is always colored, while the entostroma is always dark. The entostroma may be highly developed when in thick bark, or often there is very little entostroma and the perithecia are oriented under a very thin ectostromatic layer.

The asci are 70–80  $\mu$  long for the spore part and 65–80  $\mu$  for the stalk. The latter is very long and filiform. The ascospores are  $4-6 \times 9-12 \mu$ .

The conidial layer arises from the ectostroma as described for *H. Howeianum*. In color and dimensions of conidia no distinction was found between this species and many others. During rainy weather the conidia develop on old stromata as well as on young. This layer disappears in dry weather, leaving the ectostroma smooth.

In old stromata the ostiola are stuffed with a white mycelial growth. This is the so-called distinctive character of *H. perforatum* and supposedly separates that species from *H. rubiginosum*. The writer found this condition more frequently with pulvinate stromata, but also often in effused ones. Shear suggested to the writer that this growth is due to germinating ascospores, and this has been found to be so. Therefore, as measurements of asci and ascospores and stromal characters of *H. perforatum* agree entirely with those of *H. rubiginosum*, the two must be identical.

The most distinctive characters of this species are the color and irregular growth of the stroma. It is more apt to be confused

with the effused form of *H. multiforme* Fries. However, the perithecia are larger in the latter and the ostiolar necks are always papillate, whereas they are umbilicate in *H. rubiginosum*.

*Hypoxylon fuscum* Fries will not be described here because in development it closely approaches the other members of this series. It is distinct in that it occurs only on *Alnus*, *Corylus*, and *Betula*.

The members of this series studied here are *H. Howeianum*, *H. coccineum*, *H. fuscum*, and *H. rubiginosum*. They are alike in having an ectostroma which is some shade of red or purple, in the woody texture of the mature stroma, and in possessing umbilicate ostiolar necks—that is, the perithecial neck does not project out beyond the ectostroma. The first two species are fairly constant in their subglobose form, whether erumpent from bark or on decorticated wood. These two can be separated on ascospore characters. *H. fuscum* and *H. rubiginosum* assume a form determined by the substratum. The last two can be separated on the measurements of ascus and ascospore, and usually on color of ectostroma and on host. No distinctive conidial characters were found.

## 2. Stroma Black, Carbonaceous, Ostiola Annulate

### HYPOXYLON ANNULATUM (Schw.) Mont.

The stroma in this species is hemispherical when growing from bark and indeterminately effused when on decorticated wood. Plate I, fig. 10, shows the pulvinate type, and on Plate IV, fig. 5, is a drawing showing a longitudinal section of such a stroma. The color of the young stroma is tobacco-brown, and at maturity it is black. The apices of the perithecia are flattened into a truncate disk with the papillate ostiolar necks in the center. The perithecia are large, nearly globose, and about 1 mm. in diameter. The asci are cylindrical, sp. p. 60–70  $\mu$  in length, and with a stalk 35–40  $\mu$  long. The ascospores are uniseriate, inequilaterally elliptical, 3–4  $\times$  7–9  $\mu$ , and dark brown at maturity.

After inoculation with ascospores tobacco-brown mycelium arises on decorticated wood, or when the inoculation is made on bark, between the bark and the cortex. This coalesces to

form an ectostroma. When on wood it is loose, subiculum-like, and has the appearance of a similar structure in *Rosellinia aquila* (Fries) De-Not. When under the bark it swells and breaks the latter, becoming erumpent by the developing palisade layer of entostroma under it. The perithecia arise in the periphery of the entostroma.

The conidial layer arises from the ectostroma as a thick, brown subiculum. The conidiophores are much branched, giving rise to spores as described for the other species of *Hypoxylon*. The only distinctive character here is the thick, subiculum-like growth, and the brown color. The spores are similar to other species of *Hypoxylon*.

The texture of the mature stroma is very hard and carbonaceous. It is impossible to section this species.

*H. annulatum* occurs on members of the white oak group. The writer has found it only on *Q. alba*, *Q. stellata*, and *Q. montana*.

The point of special interest with this species is the development of the annulate depression around the perithecial necks. The drawing on Plate IV, fig. 5, shows what occurs. The developing perithecia in this *Hypoxylon* group grow through the ectostroma to the extent of the ring. The top of the perithecium breaks through and the margin of the ectostroma produces the circular depression.

#### HYPOXYLON MARGINATUM (Schw.) Berk.

The stroma at maturity is black, semipulvinate to hemispherical, very variable in form, 1–4 cm. in diameter and 2–10  $\mu$  thick. It is never flat and effused. The surface is slightly roughened by the annular depressions. The asci are cylindrical, with sp. p. 65–75  $\mu$  and stalk 40–50  $\mu$ . The ascospores are uniseriate, slightly inequilaterally elliptical,  $3\text{--}4 \times 7\text{--}9 \mu$ , and at maturity are opaque.

The young stroma is olivaceous-green inside and darker on the outside with a greenish tint. The conidial layer is also of that shade. This species never forms a thick subiculum as in *H. annulatum*. In young stages the stromata are nearly globose, with an even surface, showing no evidence of perithecia, the latter breaking through the ectostroma late in the development.



The entostroma is always well developed. From inoculations on decorticated wood no flat effused forms were gotten from *H. marginatum*, but *H. annulatum* did produce an effused form.

The conidia are produced as in other species of *Hypoxylon*, and are about  $4 \times 3 \mu$  in diameter and hyaline to greenish-hyaline.

This species occurs on a variety of hosts. The writer has found it on *Quercus velutina*, *Q. nigra*, *Q. borealis*, var. *maxima*, *Acer rubrum*, and *Betula nigra*.

Shear (27: 84) says *H. annulatum* is equal to an effused form of *H. marginatum*. After studying these closely in the field the writer has found no intergrading forms, and the differences in color of young stromata, presence of a subiculum in the former and not in the latter, differences in hosts, and constant differences of development, are certainly sufficient to maintain them as distinct species.

#### HYPOXYLON EFFUSUM Nits.

The stroma is indefinitely effused, convex, black at maturity, and dotted by the exposed annular disks as in the two previously described species. In old stromata the ectostroma wears off, leaving the perithecia exposed. The condition varies then from a thin, widely effused one, which resembles a smooth form of *Nummularia Bulliardii* Tul., to one of simple perithecia in old specimens, such as are seen in forms of *Rosellinia*. The asci are cylindrical, attenuated into a stalk. The spore part is  $50-60 \mu$  long and the stalk is  $30-40 \mu$ . The ascospores are uniseriate,  $5-8 \times 3-3\frac{1}{2} \mu$ , and at maturity are pale brown.

This species occurs in the tropics and in the Southern States.

The *H. effusum* studied here is the same as Saccardo understands it in Myc. Venet. 1470, and not the same as the Ellis specimen on *Ulmus*, N.A.F. 2114. The latter is a form of *H. serpens* Fries.

The constant characters here are the small spores, the very flat, effused stroma, developing under the bark in large sheets as in *N. Bulliardii*, and the lack of entostromatic development as found in *H. annulatum* and *H. marginatum*.

*Hypoxylon polyspermum* Mont. has been made a synonym of

*H. effusum* by Theissen (28). He separates *H. annulatum*, *H. marginatum*, and *H. effusum* in that paper on spore and perithecial measurements. The fact that all of them show the annular depression around the ostiolar papillum and have spores very close in measurements has led to much confusion.

*H. Michelianum* Ges. & De-Not. and *H. malleolus* Berk. & Rav. are black and have this ostiolar circular depression. The former is irregularly pulvinate and the latter is constantly globose to subglobose. They occur in the Southern States and in Porto Rico.

*H. serpens* Fries and *H. illitum* (Schw.) Sacc. have a carbonaceous ectostroma and sometimes an annular depression is visible around the ostiolar papilla. These two species are transitions between this series and the next.

### 3. No Entostroma, Perithecia Sunken in the Substratum, Ostiola Papillate

#### HYPOXYLON MORSEI Berk. & Curt.

In this species the stroma is 3–5 mm. in diameter, flattened above, partially erumpent, and is composed of from 5–10 perithecia. The latter are large, about 1 mm. in diameter, globose, and flattened on top. The asci are cylindrical, 120–140 sp. p., and the stalk is 40–60  $\mu$  long. The ascospores are uniseriate, elliptical, with rounded to acute ends, and measure  $17\text{--}22 \times 8\text{--}10 \mu$ . A mature stroma is shown on Plate I, fig. 6.

There is a thin ectostroma covering the perithecia and there is practically no entostroma. The bases of the perithecia are sunken in the wood.

This species occurs on *Corylus* and *Salix*.

#### HYPOXYLON GREGALE (Schw.) Berk.

The mature stroma is black, semiimmersed in the bark, slightly erumpent, hardly as much so as in *H. Morsei*, and is 2–3 mm. in diameter. The surface is smooth except for the papillate erumpent ostiolar necks. The perithecia are flask-shaped,  $\frac{1}{2}$  mm. in diameter. The asci are cylindrical, 75–80  $\mu$  long for the spore part and 20  $\mu$  for the stalk. The spores are

uniseriate, elliptical,  $7-9 \times 12-14 \mu$ , and at maturity are opaque.

This species lies near *H. Morsei*, in that the perithecia are seated directly in the wood, and so there is practically no entostroma. In both the stromata are flat, but very definite in outline.

#### HYPOXYLON UDUM (Fries) Nits.

The stroma is flat, orbicular, 4-5 mm. in diameter or less, and about 1 mm. thick. When young the ectostroma is light clay colored, varying to brown at maturity and almost black on old stromata. The young stromata are soft and fleshy and mature ones are membranaceous to woody. They are easily sectioned. The asci are  $140-160 \mu$  long for the spore part and  $25-35 \mu$  for the stalk. The ascospores are  $22-28 \times 10-12 \mu$ .

The conidia are hyaline, oblong to globose, and  $4-6 \times 4 \mu$ . The general characters of the conidial layer are similar to those found in other *Hypoxylon* species.

#### HYPOXYLON SEMIIMMERSUM Nits.

The young stroma is very similar to that of the above species. The ectostroma is first seen as a light gray, very thin layer. The perithecia arise under this in the woody tissue. The mature stroma is  $4-5 \times 2-3$  mm., and is flat with only the ostiolar papillae projecting, or individual perithecia are visible. Often the perithecia are serially arranged in the medullary crevices and are connected only by a very thin ectostroma. There is no entostroma. The asci are  $80-90 \mu$  long for the spore part, and  $60-70 \mu$  for the stalk. The ascospores are  $17-19 \times 6-8 \mu$ , and elliptical with rounded ends.

The conidiophores form a light gray layer over the ectostroma. The conidia are hyaline and  $5-8 \times 3-5 \mu$ .

This form occurs at Ithaca on decorticated wood, and has been found only on *Quercus*. Rehm determined this as *H. udum* Fries, but the spores are too small for that species. In spore dimensions it is intermediate between *H. udum* and *H. serpens*.

#### HYPOXYLON CONFLUENS (Fries)

In this species the perithecia are also seated directly in the wood, but instead of forming sharply delimited stromata, they

are practically free (PLATE 35, FIG. 7). Each perithecium is covered with an ectostroma, as in the genus *Rosellinia*. The stroma consists of from 5–10 perithecia united by their bases.

This series comprises most of the forms placed in the subgenus *Endoxylon* Nits. The constant characters are the small, flat, membranaceous stromata, the partially sunken perithecia with practically no entostroma, and the papillate perithecial necks. The last character separates these species from the effused ones in the first series, and the membranaceous texture of the stroma from effused species in the second group. The differences in ascospore dimensions are sufficient to separate the species.

#### NUMMULARIA DISCRETA (Schw.) Tul.

It is impossible to place this species in any one of the four series considered here. However, it will be discussed at this point because it appears to be intermediate between the third and fourth series, but it also has affinities for the genus *Anthostoma*.

The stromata are concave, sunken below the bark, definite in outline, and 3–5  $\mu$  in diameter. The perithecia are sunken in the stroma, and reach the surface by long ostiolar necks as in the genus *Valsa*. The asci are cylindrical, short-stalked, 160–200  $\times$  12–15  $\mu$ . The spores are globose, black at maturity, 12–14  $\mu$  in diameter, and uniseriate in the ascus.

A light-colored ectostroma composed of angular pseudo-parenchymatous cells develops between the bark and cortex. Under this a valsoid entostroma arises. This is composed of wood mixed with hyphae, and is limited by a black line. It is not a stroma in the sense of most species of *Hypoxylon*. Initial coils in this entostroma produce globose perithecia that grow outward into long necks. The latter penetrate through the ectostroma (PLATE 36, FIGS. 3, 4). Before this development is completed the ectostromatic cells swell and rupture the bark. Just as soon as the bark is broken, so air can get in, the upper surface of the ectostroma becomes covered with a hyphomycetous conidial growth. The conidia are continuous, hyaline, and are borne in the same manner as in the genus *Hypoxylon*.

Tulasne (30: Tab. 5, figs. 2 and 14) pictures the conidia in this species and in *N. Bulliardii* Tul. as arising under a definite

stromatic layer, and this illustration has been the basis for separating the genus *Nummularia* from *Hypoxylon* since his publication. As described above, the writer has found that the conidia arise on the ectostroma in the same manner as in the genus *Hypoxylon*. Cooper (4) in a letter to the writer says, "Conidia never appeared, however, except where cracks or perforations in the bark permitted the free access of air. I am quite certain that conidia were not formed before the free access of air under the blisters. At no time did I find conidia under a stromal layer."

This species with its long perithecial necks and valsoid stroma furnishes a transition towards the genus *Anthostoma*. It is quite different from other members of Tulasne's genus *Nummularia*, and also from any *Hypoxylon* species. The writer thinks it is best to leave it in the *Hypoxylon* group, rather than place it in *Anthostoma*, because there is more ectostromatic development here than in the latter genus.

#### 4. Stroma Constantly Effused, Thin, Ostiola Papillate

##### NUMMULARIA CLYPEUS (Schw.) Cooke

The stroma is black, effused for several inches to several feet, erumpent from below the bark, convex, and resembles *N. Bulliardii*. The perithecia are globose to flattened where crowded, with short ostiolar necks which penetrate the ectostroma and are papillate on the surface. The asci are for the spore part 120–140  $\mu$  in length, and 20–30  $\mu$  for the stalk. The black ascospores are uniseriate, fusoid-elliptical, 9–12  $\times$  18–22  $\mu$ . The form and measurements of the ascospores as well as the thickness of the stroma will serve to distinguish this species from *N. Bulliardii*.

As in *Nummularia* and *Hypoxylon* species of this type the ectostroma spreads under the bark by coalescing and growing laterally for several inches to several feet. The perithecia are usually fully developed before the bark ruptures. When this breaks off it carries with it the part of the ectostroma which is mixed with bark cells and leaves a hard black crust. Conidia develop from any exposed stromatic surface during rainy weather. When the conidial layer disappears there is left a remnant

around the margin. This is also characteristic of *Hypoxylon* species.

HYPOXYLON ATROPUNCTATUM (Schw.) Cooke

This fungus has been placed in *Nummularia*, as well as in *Diatrype*. In development it certainly belongs along with *N. Bulliardi*.

The stroma is white, punctate with black ostiolar papillae, and widely effused. The perithecia are globose to flask-shaped, 200–300  $\mu$  in diameter. The asci are 150–160  $\times$  12–14  $\mu$ , with very short stalks. The ascospores are uniseriate, 25–30  $\times$  11–14  $\mu$ , at maturity black, and are elliptical with pointed ends. The paraphyses are filiform.

This species is very common on oaks in the Southern States.

The ascospore sends a germ tube into the bark which ramifies and develops a wide mycelial plexus, which coalescing with other infections produces a layer several feet in length. A black circumscribing line delimits each stroma (PLATE 39, FIGS. 1, 2, 3).

When sufficient ectostroma is formed between the bark and cortex it swells and bursts the bark in longitudinal cracks. As soon as the air enters, conidiophores arise from the upper surface of the exposed ectostroma. The bark now falls off in large strips, exposing the gray conidial layer. The conidiophores produced in cracks of the bark are very long and branched and produce masses of globose conidia about 5–6  $\mu$  in diameter. This species is remarkable in that the pulverulent conidial layer is found all during the summer and does not disappear in dry weather as is the case with other species of *Hypoxylon* (PLATE 38, FIGS. 1, 2).

The entostroma is very thin, and the perithecia are almost in contact with the wood. The upper surface is covered with the white ectostroma. This layer is very hard and carbonaceous (PLATE 35, FIG. 4, and PLATE 38, FIG. 4).

In very old stromata the white layer on top peels off entirely or in patches, leaving a black entostroma exposed. Then this species is confused with *H. stigmatum* Cooke and is probably identical with it.

## NUMMULARIA PUNCTULATA (Berk. &amp; Rav.) Sacc.

The stroma here is widely effused as in the preceding one, developing under the bark, and at maturity throwing it off in wide sheets. Most of the ectostroma peels off at maturity, leaving the surface smooth and shining as if glazed. The perithecia are globose to flask-shaped. Very few of them produce asci. The latter are small, cylindrical, and the spore part is  $45-55\ \mu$  and the stalk is  $30-40\ \mu$ . The ascospores are light brown, uniseriate, elliptical,  $3-4 \times 7-9\ \mu$ . The peculiarly constant character in this species is the fact that the ostiolar necks rarely grow entirely through the ectostroma. They resemble needle pricks in a smooth surface.

The conidia develop on any exposed stromata during rainy weather. The spores are hyaline and  $3-3\frac{1}{2} \times 6-8\ \mu$ .

The writer placed pieces of this species in a moist chamber and after several weeks the surface of the stroma was covered with papillae just as in other species of *Nummularia*. When sectioned this material revealed all the perithecia filled with asci. From this it would seem that the perithecial necks failing to come through is correlated with a lack of ascus development.

*Nummularia tinctor* (Berk.) Ellis & Ev., *N. hypophloea* (Berk. & Rav.) Cooke, and *N. microplaca* (Berk. & Curt.) Cooke were also studied in the field and in the laboratory, and as they develop in the same manner as the ones described above they will not be discussed further.

As the writer has stated previously, the one difference between the genus *Nummularia* and *Hypoxylon* according to Tulasne is the substromal conidial layer in the former and not in the latter. Now that the writer has shown that the conidial layer arises in the same manner in both genera, it is only logical to place the species that were taken out of *Hypoxylon* and placed in *Nummularia* back in *Hypoxylon*. To maintain the genus *Nummularia* there should be at least one difference which is constant for the majority of species in the two groups. The writer knows of no such difference. The majority of forms now included in *Hypoxylon* are effused as often as they are pulvinate. The globose or hemispherical forms are exceptional.

The members of this series are characterized by being con-

stantly flat and effused, with very little entostromatic development, but the perithecia do not rest directly in the wood. In texture the stroma is always carbonaceous, and so it is impossible to section. The ostiolar necks are papillate in all the species studied except *N. discreta* and *N. punctulata*. The writer considers the former a transition form to *Anthostoma*, and so not a good member of this group. *N. punctulata* has papillate ostiolar necks when there is ascal development, and so should be included here.

Effused forms of *H. rubiginosum*, such as those which have been named *H. suborbiculare* Peck and *N. lateritia* Ellis, have the same type of development.

#### ROSELLINIA Ces. & De-Not.

In this genus the characters of the perithecial centrum are exactly the same as found in the Xylariaceae. It was placed in the Sphaeriaceae by Lindau (16: 394), because he thought the perithecia were free, that is, superficial and not stromatic.

#### ROSELLINIA AQUILA (Fries) De-Not.

Inoculations of wood of *Carpinus caroliniana* were made in Feb. 1926, and the development was studied. A definite ectostroma forms in the bark and ruptures it. This gives rise to a subiculum of brown hyphae which grows out through the ruptures and, after coalescing, forms a more or less indefinite mat on the outside of the bark. Conidia arise on this subiculum in the same manner as in the genus *Hypoxylon* (PLATE 37, FIGS. 1, 2, 3, 4). Next a well-developed entostroma, composed of elongate parallel hyphae, forms under the ectostroma. In its periphery one or more perithecial initials develop. The growing perithecium pushes up the ectostroma, the subiculum disappears, and the mature perithecium is tightly fitted within an outer ectostromatic layer (PLATE 36, FIG. 8). Ellis (6: 163) says there is an outer, rather thick but brittle carbonaceous wall, and an inner coriaceous one. The perithecial neck grows through the ectostroma as in *Hypoxylon*.

The conidiophores are irregularly branched. Apical conidia are cut off, then the hypha elongates from under the conidium



in a vertical direction and produces another conidium, and so on (FIG. 4). The conidiophores and conidia are greenish-brown in color, oblong, and variable in size,  $2-3 \times 5-8 \mu$ .

*Rosellinia subiculata* (Schw.) Sacc. develops in exactly the same manner as *R. aquila*. PLATE 37, FIG. 5, shows a mature perithecium oriented under an ectostroma. The subiculum has disappeared. In this species there is no well-developed entostroma.

The perithecia of *Rosellinia Clavariae* (Tul.) Wint. (PLATE 37, FIG. 6) differ from those of most other *Rosellinia* species in that there is no entostroma and the ectostroma at maturity forms a tight coat on the outside of the perithecial wall. This condition is fully equal to an illustration of *Sordaria* sp. by Gwynne-Vanhan (11: 138, fig. 100).

Forms now placed in the genus *Rosellinia* have the same type of development as those in the genus *Hypoxylon*. Also they are definitely connected with the genus *Hypoxylon* by such transition forms as *H. confluens* and *H. annulatum*. However, as long as the majority of *Rosellinia* species have single perithecia in the stroma and the majority of *Hypoxylon* species have many, it is desirable to separate these two groups; that is, to continue them as separate genera, but they should certainly be in the same family.

#### USTULINA Tul.

This genus was founded by Tulasne (30: 23) on *Hypoxylon ustulatum* Fries and was segregated on account of its fleshy conidial layer.

A longitudinal section through a young stroma of this species is shown in PLATE 36, FIG. 5. The conidial layer forms on the outside of the ectostroma (FIG. 5b). At this stage young perithecial initials appear (FIG. 5c). After further growth the perithecia come to occupy most of the stroma, the conidial layer disappears, and the ectostroma remains as a black, carbonaceous layer over the perithecia. The perithecial centrum characters are similar to what is seen in *Hypoxylon*.

The development in this genus is exactly the same as in *Hypoxylon*. The fleshy texture of the young stroma is not limited to *Ustulina*, as all *Hypoxylon* species are fleshy when

young. The gray color of the immature stroma is not a character of generic importance, as *Hypoxylon* species in this stage vary from white to shades of red, purple, and so on. If the color, or the form of the stroma, is sufficient to maintain the genus *Ustulina*, then the present genus *Hypoxylon* could with equal propriety be broken up into as many genera as there are now species. Therefore, it is suggested that members of this genus be placed back in *Hypoxylon*.

#### DALDINIA De-Not.

Forms of *D. concentrica* (Bolt.) Ces. & De-Not., *D. Eschscholzii* (Ehr.) Rehm, *D. vernicosa* (Schw.) Ces. & De-Not., and *Hypoxylon placentifforme* Berk. & Curt. have been studied.

A grayish-brown ectostroma becomes erumpent through crevices in the bark, or when on decorticated wood this stromal growth occurs irregularly effused on the surface. Under the ectostroma a dark-colored entostroma soon develops, pushing the former upward. Sections of young stromata 1 to 2 mm. thick show a peripheral layer of perithecial initials oriented just under the light-colored ectostroma. At this stage the condition is identical with that found in such *Hypoxylon* species as *H. coccineum* and *H. Howeianum*. There is no indication of concentric zones under the perithecial layer. By studying a series of forms, beginning with one with one perithecial layer and ending with one with many concentric zones (PLATE 36, FIG. 1), the writer found that the first series of initials disintegrates and meristomatic hyphae from below grow upward and around these coils and produce another series of initials. This action continues until from six to forty such zones are formed. Finally, when the last initials develop into perithecia the zonation ceases. The concentric zones then in *Daldinia* are apparently formed from perithecia which are arrested in their development and disintegrate.

All of the *Daldinia* species mentioned above have certain characters in common besides the concentric zonation. The conidial layer is a light grayish-brown in all four species. The conidia are hyaline, with a slightly greenish tint, and are 6-8  $\times$  4-5  $\mu$ , and are borne on sympodially branched conidiophores.

The color of the mature ectostroma, after the conidial layer disappears, is a dark purple. The perithecial necks do not penetrate through the ectostroma, so the ostiola are umbilicate. The ascus and ascospore measurements are very close together in the four species. The differences are found in the forms of the stromata. *D. concentrica* is usually sessile and centrally attached, varying to forms that are plainly stipitate. *D. vernicosa* is laterally compressed, plainly stipitate, and tends to become hollow inside, due to gelatinization of parts of the entostroma. *D. Eschscholzii* is similar to *D. concentrica* in all points except size. It is usually much larger than the latter and tends to spread out at the base. *H. placentifforme* (this is a true *Daldinia*) develops an irregular flat stroma with forms running into *D. Eschscholzii*.

The development in the above species in all fundamental characters is sufficiently similar to that in *Hypoxylon* to indicate a very close relationship, but the concentric zonation is so striking that it would appear best to continue the genus *Daldinia*.

#### ANTHOSTOMA Nits.

This genus is placed in the Valsaceae by Lindau (16: 455). He separates it from *Valsa* on spore characters. In *Anthostoma* the spores are brown and not allantoid. The stromatic characters are the same in the two genera.

Nitschke (19: 110) separated the genus into two subgenera, Euanthostoma and Lopadostoma. In the first he places species which are effused and usually inhabit decorticated wood, and in the second subgenus he places clustered forms.

Wehmeyer (31: 638) emends the genus *Anthostoma* and places it in von Höhnelt's family Allantosphaeriaceae. He says the perithecia are separately erumpent (Euanthostoma), or clustered and collectively erumpent (Lopadostoma). The ostiolar necks are irregularly sulcate or merely punctate. The spores are brown, allantoid to cylindrical or usually inequilaterally elliptical and one-celled.

In *Anthostoma gastrinum* (Fries) Sacc. the writer finds the perithecia are single or in valloid groups in long lines in cracks in the bark (PLATE 38, FIG. 9). The asci line the base and sides

of the perithecium. They are cylindrical, stalked, with uniseriate, dark brown ascospores. The paraphyses are thread-like and similar to those in *Hypoxylon*. Periphyses line the ostiolum.

*Anthostoma grandineum* (Berk. & Rav.) Sacc. occurs on oak bark (PLATE 38, FIG. 6). The perithecia are in a definite stroma and the development is similar to that of *Hypoxylon*. This species differs from most *Hypoxylon* species only in its microscopic proportions. It should be in the genus *Hypoxylon*.

*Anthostomella minor* (Ellis & Ev.) (PLATE 38, FIG. 8) exhibits a so-called clypeus, which in this case is due to a combination of ectostroma between the outer cells of the host and a blackening of these cells. There is no definite stromal plate surrounding the perithecial necks. Both ectostroma and entostroma are poorly developed as in the members of the genus *Anthostoma*. The perithecial centrum characters are similar to *Anthostoma* and *Hypoxylon*. The only difference between members of this genus and those of *Anthostoma* is a tendency for the perithecia to be isolated in the former and aggregated in the latter. The writer thinks this tendency is not sufficiently striking to maintain them as separate genera, and suggests that the species now in *Anthostomella* be placed in *Anthostoma*.

The writer also thinks the genus *Anthostoma* should be placed in the Xylariaceae. The perithecial centrum characters are exactly the same as members of the genera *Hypoxylon*, *Xylaria*, etc. The difference is entirely a difference in the amount of stroma present. Here the ectostroma is hardly sufficient to rupture the bark, and this is accomplished by the upward growing perithecial necks. The entostroma is also poorly developed, being valsoid in that it is mixed with wood. Members of this genus closely approach such *Hypoxylon* species as *H. udum*, *H. semiimmersum*, and *N. discreta*. In all of these the perithecia are seated directly in the wood, with only a thin ectostroma uniting them.

Petrak (23: 253) says *Anthostoma* stands in no close relationship with genuine *Valsa* species, and that the centrum cannot be distinguished from that of *Rosellinia* nor *Hypoxylon*. Further, he says that *Rosellinia*, *Creosphaeria*, *Leptomassaria*, *Antho-*

*stomella* and *Anthostoma* stand in a genetical series with the Xylariaceae and truly form a sharply characterized group through the form of the centrum and spores. Also he says there is no sharp distinction between *Anthostoma* and *Anthostomella*.

The species of *Anthostoma* represent the most primitive forms in point of stroma in the Xylariaceae, and probably are transition forms between this family and the Allantosphaeriaceae.

### Discussion

These developmental studies show most strikingly that the members of the dark-spored series have many characters in common. In spite of this, many of these genera have been placed in widely separated families on stromal characters alone by such investigators as Nitschke (19), Cooke (3), Lindau (16), and Ellis (6). Orton (21: 50) says: "The presence or absence of stroma has been long regarded as a basis for the delimitation of systematic groups of major rank, but it seems clear to me that with increasing knowledge this usage is quite indefensible since in many cases it separates what are obviously on phylogenetic grounds closely related species." Von Höhnelt (13, 14) uses many characters in the separation of major groups, and lays particular emphasis on those of the perithecial centrum. Further, von Höhnelt separates his families, Allantosphaeriaceae and Diaporthaceae, entirely on characters found in the perithecial centrum. The proof of the continuity of any group is whether the members have more characters in common with each other than with other forms, and the presence of numerous transitions which would tend to make it difficult to satisfactorily delimit genera.

In summarizing the characters common to this series they will be considered under: (1) The stroma, (2) development of the perithecium, (3) the perithecial centrum, and (4) the conidial fructification. The differences will also be discussed under these heads. They will be differences in the amount and form of stroma, in ascus and spore measurements, and in the relation of the stroma to the substratum.

1. **Stroma.**—The minimum stromatic development is found in the genus *Anthostoma*. The perithecia in this genus are never

encased in such a thick stromatic wall as most of the forms in *Rosellinia*, *Hypoxylon*, etc. If the stroma is to be considered as indicative of phylogenetic development in this series, then *Anthostoma* is the most primitive, followed by *Rosellinia*, with transitions through *Rosellinia aquila*, into *Hypoxylon confluens*, *H. annulatum*, *H. semiimmersum*, *H. udum*, and so to ones with much entostroma such as *H. Howeianum*. Then from other *Hypoxylon* species such as *H. leucocreas*, which is white inside but patellate, we can go by a perfect series of transitions into *Xylaria*. The transitions in this series are so complete that any genera erected on the amount or form of the stroma will be only approximate.

This condition has been recognized by many investigators. Theissen (29), in discussing the question of species grouping in, the Xylariaceae, shows the uncertainty of separation between *Xylaria* and *Hypoxylon*, and says the single separation character is the stalk formation, which in itself alone in other fungous groups is not sufficient for generic separations, e.g., Pezizales, and even with the Xylariaceae as in *Daldinia* (some are stalked and some are not). The proof for this is in the presence of transition forms, from the central attachment and pseudo-stipitate constriction of the lower side of the stroma up to the plain stalk formation in different species and even in the same species; as for example with *Xylaria obovata*, *X. anisopleura*, *X. allantoidea*, *X. apiculata* and others. The genus *Daldinia* is united with *Hypoxylon* through *H. Petersii*, *H. exsurgens*, *H. placentifforme* and others. Even *H. coccineum* and *H. Howeianum*, which are considered typical of *Hypoxylon*, show a plain concentric zonation in thin sections.

2. **Development of the Perithecium.**—In all the forms studied here, that is, in *Rosellinia*, *Anthostomella*, *Anthostoma*, *Hypoxylon*, *Daldinia*, *Ustulina*, and *Nummularia*, the perithecia are initiated by a coiled hypha, which on further growth develops a definite wall and "Woronin hyphae" on the inside. Elements of this wall grow upward, producing the perithecial neck. Brown (1) observed this development in *Xylaria*, and Dawson (5) in *Poronia*. In most of the species the perithecia are globose to flattened when crowded with very short papillate necks. The exceptions

are to be found in *Nummularia discreta* and *Anthostoma* species which have elongate necks, and in *Camarops* species which have elongate, compressed perithecia.

3. **Perithecial Centrum.**—The inside of the perithecium at an early stage is filled with "Woronin hyphae" which come to lie on the inner face of the wall. Then from this wall layer there arise paraphyses and later asci. Also periphyses arise from cells lining the wall of the neck. There is no pseudoparenchyma inside of the wall. At maturity then the perithecial centrum exhibits asci and paraphyses lining the base and sides of the wall and periphyses in the ostiolum. The asci are cylindrical and thin walled. The ascospores vary from light brown to black and are uniseriate in the ascus. They have a hyaline depression in the exospore, through which the germ tube is extruded. The shape of the ascospores varies from fusoid to inequilaterally elliptical to globose.

4. **Conidial Fructification.**—The conidia in species studied here are borne on hyphomycetous conidiophores, developing sympodially, singly or in clusters. All conidia are unicellular and very small. The conidiophores arise on an ectostroma which develops before the perithecia. The differences in conidia are not marked. In *Rosellinia* they are a little larger and darker than in *Hypoxylon*. In the latter genus very few differences in conidial dimensions were found which would aid in species determination. However, there are differences in color of the conidial layer and underlying ectostroma in a few species. *Xylaria* according to Brown (1) and *Poronia* according to Seaver, Whetzel, and Westcott (26) have the same general type of conidial development as the forms studied by the writer.

### The Question of Generic Separations

In the Xylariaceae according to Lindau (16: 480) the generic separations are based on the form and texture of the stroma in all cases except for *Nummularia*, which was supposed to have conidia arising under a stroma, and *Camarops* and *Xylobotryon*, which should have two-celled ascospores. The writer has shown that the conidia in *Nummularia* develop in the same manner as in *Hypoxylon*. *Camarops* has been shown to have one-celled

ascospores by Theissen (29) and to equal *Bolinia*. *Xylobotryon* was examined by the writer and it has two-celled ascospores which are biserial in clavate asci, and so clearly does not belong in this series. Theissen (29) sees no genetical differences between *Hypoxylon* and *Ustulina*, and says in *Ustulina* the form of the conidial layer furnishes no basis for generic separation. Lindau (16: 481) separates these two genera as follows: "... young stroma from the beginning thick and fleshy, covered with a conidial layer, at maturity carbonaceous . . . *Ustulina*; stroma from the beginning woody or carbonaceous . . . *Hypoxylon*." Theissen says further that this principle cannot stand in the face of so many *Hypoxylon* species which in the young condition are fleshy. The writer has found the stroma in all species of *Hypoxylon* studied to be just as fleshy when young as that of *Ustulina*. So this genus should be merged with *Hypoxylon*. This leaves the separations of the remaining genera to be based on the characters of the stroma.

There are no conidial characters nor perithecial centrum characters which are common to groups of species. The differences in stromal development will represent only the most striking tendencies, and so these will not make distinct separations. The most obvious fact about all the forms in this series is the great variability of the stroma to the environment. When on bark many *Rosellinia* species are immersed, and when on hard decorticated wood they are superficial. Also one often finds two or three perithecia in the stroma. This is not done by individuals coalescing but by several perithecia developing in the single stroma as shown previously for *R. aquila*. All *Hypoxylon* species arise from under the bark when there is any; otherwise they are entirely superficial. Most of them are effused on decorticated wood, and pulvinate to hemispherical when erumpent from bark. *Hypoxylon rubiginosum* is probably the most common species. Its environmental forms have been made the basis for many species found over the world. The transitions between *Hypoxylon* and *Xylaria* are now placed in the genus *Penzigia*. That procedure causes much confusion, because these forms are not constant for any one type. *Xylaria anisopleura*, as seen by the writer, varies in any one collection from globose-



sessile to slightly stalked to forms 2 or 3 cm. high, which are plainly stalked. The much branched condition seen in *Thamnomycetes* is common only in a lesser degree in many *Xylaria* species. So, as there are no constant stromal characters to separate genera the separations will have to be based on the most striking developmental tendencies. The writer suggests the following limitations of genera.

**Anthostoma** will be limited to all forms which contain perithecia with very little stroma, single or aggregated, which are sunken in the substratum. This genus will also contain species formerly placed in *Anthostomella*.

**Rosellinia** will be limited to forms with single perithecia which are superficial. In the genus *Sordaria* are many forms which should properly belong here. Most of them show no deviation from the illustration of *Rosellinia Clavariae* (PLATE 37, FIG. 6). The thickness or thinness of the stromatic layer on the outside of the wall is certainly not sufficient reason to exclude forms that have the Xylariaceae type of perithecial centrum. Also in the genus *Melanospora* and *Neurospora*, now in the Hypocreales, there are forms that are undoubtedly more closely related to *Rosellinia* than to the other members of the families in which they stand at present. They should be merged with *Rosellinia*.

**Daldinia** will be limited to pulvinate to globose forms, which are dark inside, and in which the stroma is conspicuously zonate.

**Hypoxylon** will be limited to effused to globose forms, which are not stipitate and not conspicuously zonate. This genus will include forms now in *Nummularia* and *Ustulina*, as well as all of those in *Hypoxylon* as it is now conceived.

**Xylaria** will be limited to forms with a stalked, clavate to globose stroma, which is usually white inside.

The other genera of the Xylariaceae have sufficiently distinct characters of the stroma to be easily segregated.

The writer proposes the following emended diagnosis for the family Xylariaceae.

#### XYLARIACEAE *emend.*

Asci long cylindrical, stalks long and filiform, or short, and asci almost sessile. Asci lining the bases and sides of the peri-

thecia. Ascospores unicellular, light-brown to black, inequilaterally elliptical, to fusoid, to globose, uniseriate in the ascus. Paraphyses threadlike, branched, completely filling the perithecial cavity at an early period, and more or less gelatinizing at maturity.

Perithecia membranaceous, seated under a more or less well-developed ectostroma, with the bases in entostroma.

Conidiophores hyphomycetous, covering an exposed ectostroma, branched; conidia single or in clusters, minute, borne apically and becoming lateral by continued growth of the conidiophore.

#### KEY TO THE GENERA

Perithecia single or aggregated.

Superficial.....*Rosellinia*.

Sunken in the substratum.....*Anthostoma*.

Perithecia several to many in the stroma.

Stroma effused, pulvinate to globose and sessile.

Internal zonation prominent.....*Daldinia*.

Not conspicuous.

Perithecia elongate and strongly compressed.....*Camarops*.

Not elongate nor compressed.....*Hypoxylon*.

Stroma stalked, branched or not.

Stroma not flattened above.

Sterile portion of stroma not formed of strands.

Stroma deeply indented at the apex, bearing the perithecia in the infolded upper portion.....*Camillea*.

Stroma otherwise.

Stromata capitate, crowded.....*Kretzschmaria*.

Stromata clavate to cylindrical branched....*Xylaria*.

Sterile portion of stroma formed of strands, fertile

portion clavate.....*Thamnomycetes*.

Stroma broadened at the apex to form a flat shield-like

termination.....*Poronia*.

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## EXPLANATION OF PLATES

### PLATE 35

- Fig. 1. *Hypoxylon Howeianum* Peck.  
 Fig. 2. *Hypoxylon fuscum* Fries.  
 Fig. 3. *Nummularia discreta* (Schw.) Tul.

- Fig. 4. *Hypoxyton atropunctatum* (Schw.) Cooke.  
 Fig. 5. *Hypoxyton rubiginosum* Fries forma *fuscopurpureum*.  
 Fig. 6. *Hypoxyton Morsei* Berk. & Curt.  
 Fig. 7. *Hypoxyton confluens* (Fries).  
 Fig. 8. *Hypoxyton rubiginosum* Fries.  
 Fig. 9. *Hypoxyton rubiginosum* Fries forma *perforatum*.  
 Fig. 10. *Hypoxyton annulatum* (Schw.) Mont.

## PLATE 36

Fig. 1. Longitudinal section through a mature stroma of *Daldinia concentrica* (Bolt.) Ces. & De-Not.

Fig. 2. *Hypoxyton Howeianum* Peck. Longitudinal section showing tendency to concentric zonation.

Fig. 3. *Nummularia discreta* (Schw.) Tul. Longitudinal section through an immature stroma showing an ectostromatic layer between the periderm and the wood. The perithecia are seated directly in the wood.

Fig. 4. *Nummularia discreta* (Schw.) Tul. Section through a mature stroma after the periderm has been thrown off.

Fig. 5. *Ustulina vulgaris* Tul. Longitudinal section through an immature stroma. *a*, conidial layer. *b*, ectostroma. *c*, periphery of entostroma in which perithecia are developing.

Fig. 6. Ascogenous hypha of *Hypoxyton Howeianum* Peck.

Fig. 7. Conidiophores of *Hypoxyton Howeianum* Peck, showing sympodial growth habit.

Fig. 8. *Rosellinia aquila* (Fries) De-Not. Longitudinal section through a mature stroma. *a*, ectostroma. *b*, entostroma. *c*, perithecial wall.

## PLATE 37

Development in the genus *Rosellinia*.

Fig. 1. *Rosellinia aquila* (Fries) De-Not. Longitudinal section through an immature stroma showing conidial layer rising from an ectostroma, and a well developed entostroma with perithecial initials in the periphery.

Fig. 2. Longitudinal section showing further development of the above.

Fig. 3. Longitudinal section through a mature perithecium. *a*, ectostroma. *b*, perithecial wall. *c*, conidial layer beginning to disappear. *d*, entostroma.

Fig. 4. Drawing of conidiophores and conidia of *Rosellinia aquila*.

Fig. 5. Longitudinal section of mature stroma of *Rosellinia subiculata* (Schw.) Sacc. The perithecium is seen to have developed under a very definite ectostroma with very little entostroma.

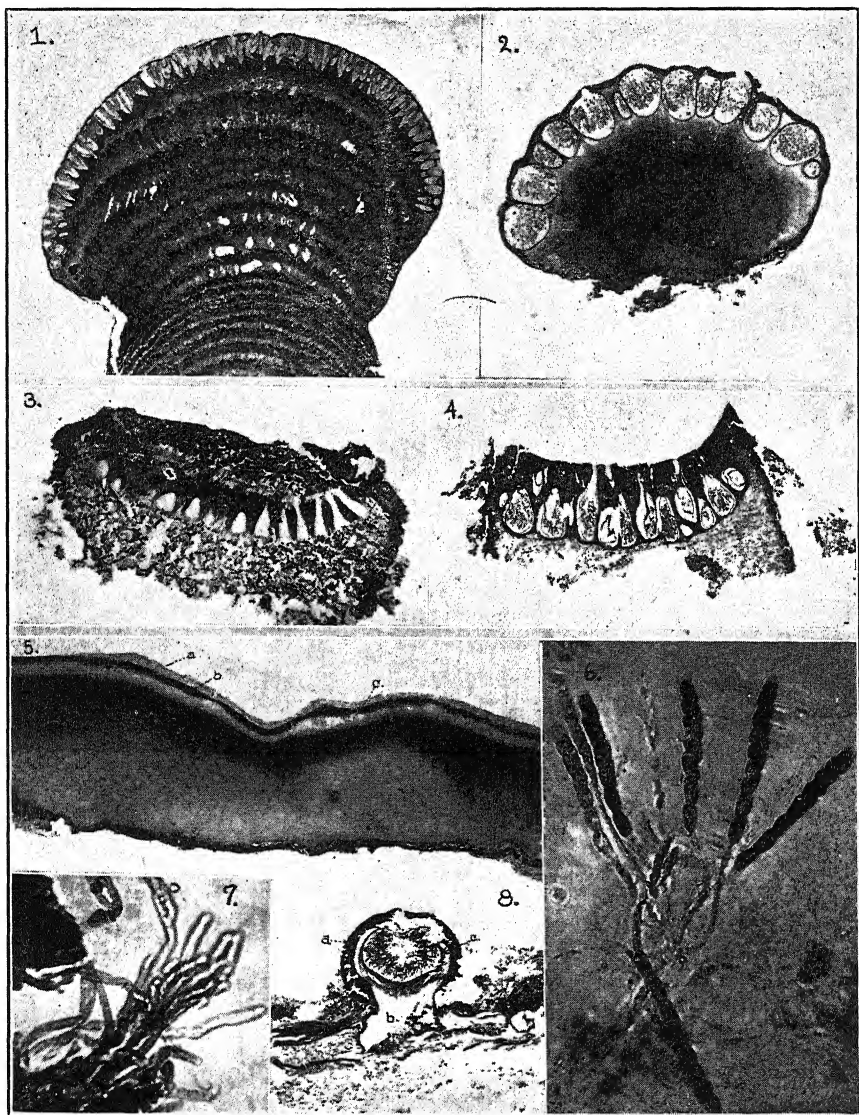
Fig. 6. Longitudinal section through a stroma of *Rosellinia Clavariae* (Tul.) Wint. This mature perithecium is covered with large cells, some of which give rise to spines. This is the remnant of the ectostroma.

## PLATE 38

Fig. 1. *Hypoxyton atropunctatum* (Schw.) Cooke., showing ectostroma, covered with conidiophores, developed under the ruptured bark.

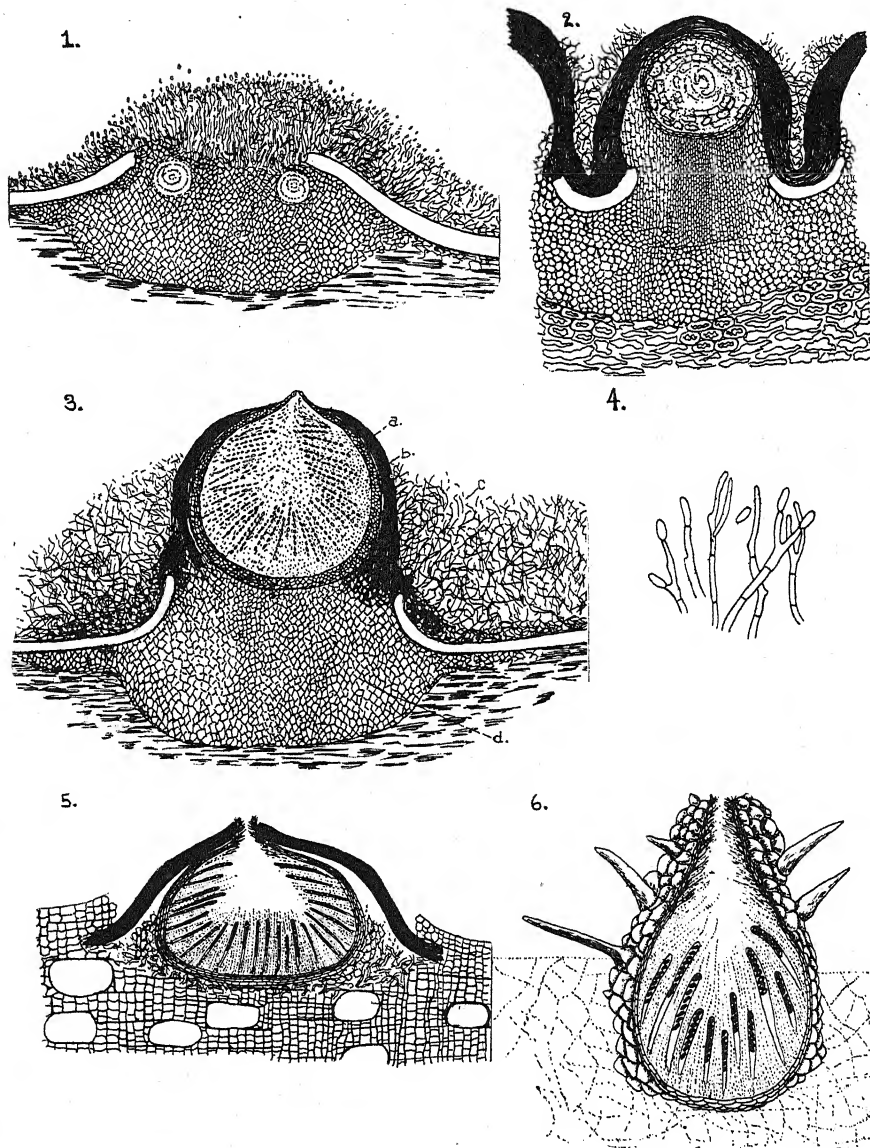
Fig. 2. Conidiophores and conidia of the above.

Fig. 3. *Hypoxyton annulatum* (Schw.) Mont. Conidiophores and conidia.



XYLARIACEAE

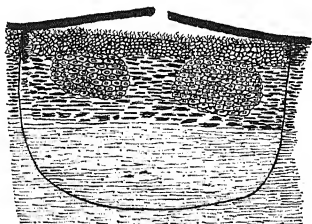








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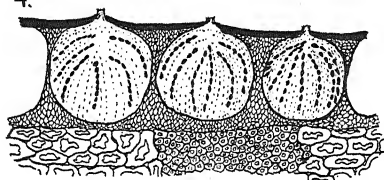
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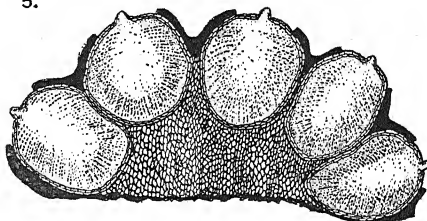
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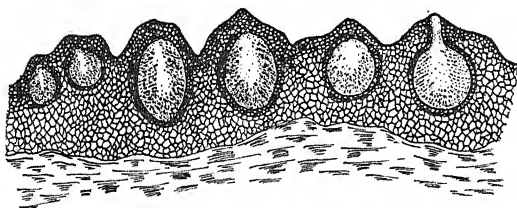
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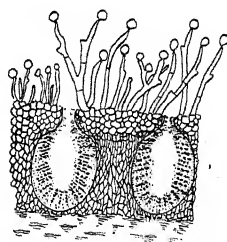
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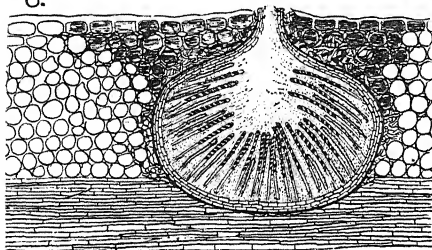
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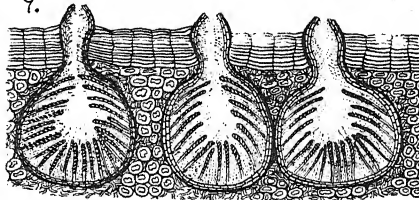
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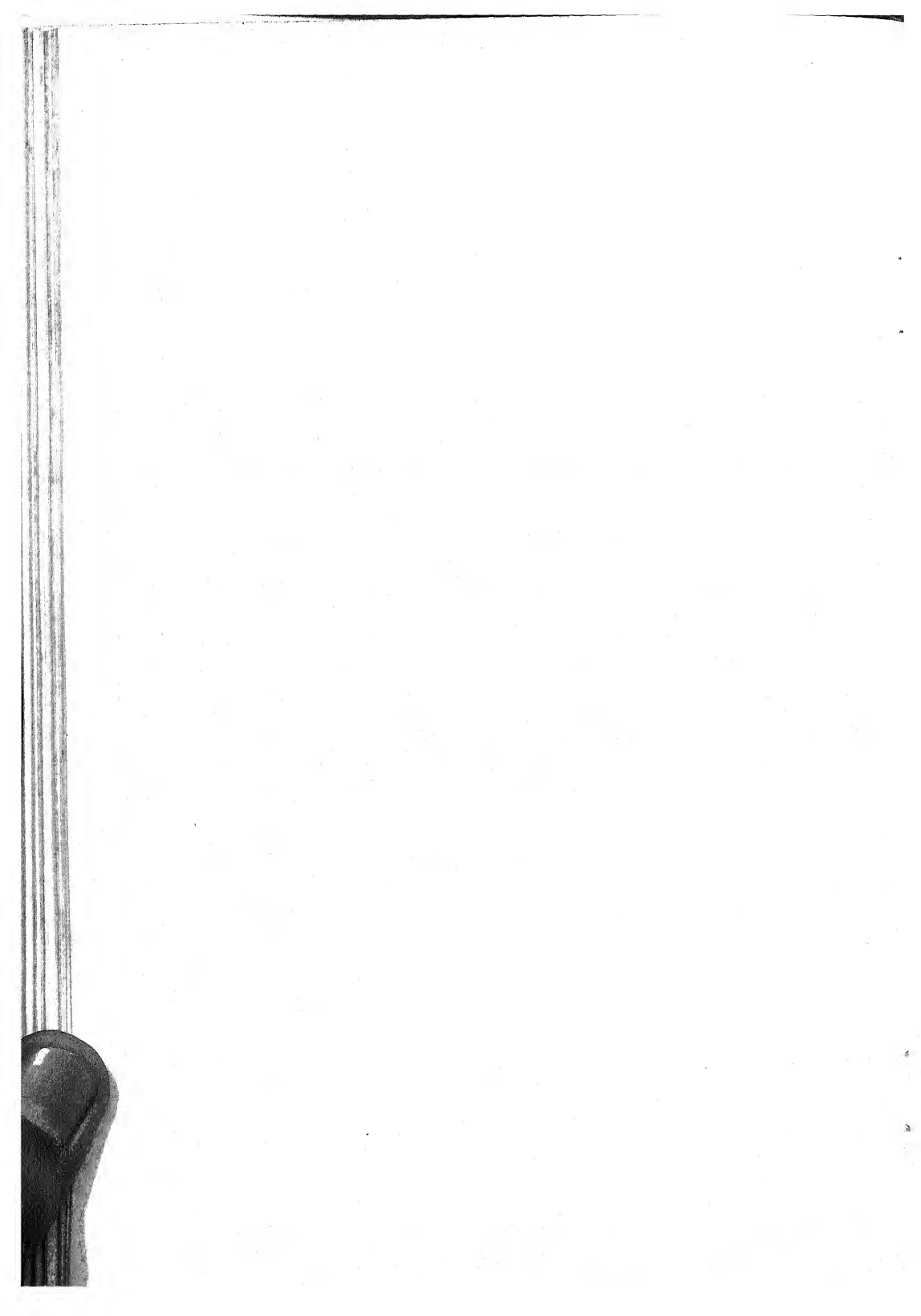


Fig. 4. *Hypoxyton atropunctatum* (Schw.) Cooke. Section through a stroma showing habit of perithecia.

Fig. 5. *Hypoxyton annulatum* (Schw.) Mont., showing the cause of the annulate depression around the ostiolar neck.

Fig. 6. *Anthostoma grandinea* (Berk. & Rav.) Sacc. Longitudinal section through a mature stroma.

Fig. 7. *Nummularia punctulata* (Berk. & Rav.) Sacc. Longitudinal section showing conidiophores growing on mature stroma.

Fig. 8. *Anthostomella minor* Ellis & Ev. Longitudinal section showing the blackening around the ostiolar necks, the so-called clypeus.

Fig. 9. *Anthostoma gastrinum* (Fries) Sacc. Longitudinal section through several perithecia showing habit.

## SOME OBSERVATIONS ON THE GERMINATION OF THE SPORES OF SOME SPECIES OF MYCETOZOA

W. R. IVIMEY COOK AND MISS E. M. HOLT

This work was commenced in order to investigate the germination of the spores of some species of Mycetozoa under artificial conditions. It had been generally found that germination is very variable, and that no certainty could be attached to the possibility of obtaining swarm spores when they are required for class work.

The statements found in Lister (5) and elsewhere, that the spores of various species germinate readily, could not be confirmed, and it was felt that certain physiological conditions must be necessary in order to achieve the results published.

During the autumn of 1927 sporangia of several species of Mycetozoa were collected in quantity from varying localities, and some preliminary experiments were carried out to test the time which must be allowed for germination to take place. It was at once obvious that this time factor differed very considerably in the species under observation. In *Reticularia Lycoperdon* germination occurred in about an hour, while in some species of the genus *Trichia* no germination was obtained in much under a week. Most species produce their sporangia in the winter when the temperature is low, on the other hand *Lycogola epidendrum* is found in a fruiting condition more or less commonly throughout the year. Experiments were therefore tried to determine what effect an alteration in the temperature, at which the germination was conducted had upon the number of swarm spores produced and the rapidity with which they appeared. The effect of light and darkness was also tested.

Some importance was also attached to the locality from which the material was collected; it was felt probable that some difference might be expected in the rate of germination in samples collected from different parts of the country. The number of varieties examined was to a large extent regulated by the quantity

of available material. In order to complete one series of experiments a very considerable number of sporangia was needed; it was therefore not possible to carry out experiments except upon those which could be collected in quantity. Five species were selected, and in four of these two separate series of germinations were used with material from different localities.

#### METHODS

The solutions in which germination was tried were selected from the results of the preliminary experiments. In general it was found that sugars and proteins were most suitable, though in addition extracts from natural substances upon which Mycetozoa are frequently found in nature were tried. Finally a series of 10 solutions was selected, which gave a fair range as regards their nutrient qualities.

The solutions finally adopted were, water, glucose 2.0 per cent and 1.0 per cent, starch 1.0 per cent, sucrose 0.3 per cent and 0.15 per cent, peptone 2.0 per cent, Knop's solution and extracts of holly leaves and pea seeds. For use about 50 cc. of the solution were placed in a petri dish about two inches in diameter, into this a sufficient quantity of spores was added to form a more or less even film over the surface, the spores were stirred in for some time in order to get them thoroughly moistened with the solution. With dry spores this wetting of the spore coat is a matter of considerable difficulty, but unless this is done the spores do not germinate. Three series of solutions were set up at the same time except in the case of *Reticularia Lycoperdon* where the rate of germination was very rapid.

Those placed in the light, were put in front of the laboratory window, and a record of the temperature was kept. Those in the dark were placed in a cupboard also in the laboratory and at the same temperature as those in the light. For those which were to be subjected to both darkness and heat an incubator was employed, and the temperature was maintained at 29° C.

Observations were made at frequent intervals, the time depending upon the expected interval before germination. The observations on the spores of *Reticularia* were commenced very soon after the cultures were set up. Since it was impossible to

have all the cultures under observation at the same time an interval of about five minutes at least had to elapse before a culture could be examined for a second time. The records of the times in *Reticularia Lycoperdon* were, therefore, necessarily somewhat inaccurate. In the case of those which took a considerable time to germinate they were examined at intervals of a few hours from the time when, judging from preliminary experiments germination was to be expected. Germination in *Trichia varia* however, was so slow that the observations were made each morning and evening only. Cultures were allowed to run for a considerable time after the first indications of germination in any solution was observed, and if at the end of that time no germination had taken place it was assumed that that solution was unsuitable for spore growth.

In some cases the germination was represented by a percentage. This was taken from several mounts of the spores made by placing a small drop of the solution with the spores in suspension upon the slide and counting those which had germinated and those which had not. Such a method was rough and only represented an approximation of the proportion of the spores which had germinated and were visible in the field. Care, however, was taken not to include in the drop any spores which had not become wetted by the solution. If any were observed in the field, they were neglected; it was found easy to recognise such spores by the shrunken appearance of the spore coat.

## RESULTS

The results of the cultures are represented by the following tables, showing the germination and time. In Tables 1, 2, 3 and 5 two distinct series of experiments were made at different times and these can be compared together.

Table 1 shows the germination of *Reticularia Lycoperdon*. It will be seen that germination was obtained in six out of the ten solutions tried; in the case of 0.3 per cent sucrose, germination was obtained in the only instance when it was tried and since successful spore germination was obtained in the weaker solution it is to be expected that the same result would have been obtained in darkness and in dark and heat as was found in light. No

TABLE 1

*Reticularia Lycoperdon*

Solutions used.	Light.				Dark.				Dark and Heat.			
	Sept. 22		Sept. 29		Sept. 21		Sept. 29		Sept. 21		Sept. 29	
	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.
Water.....	+	82	+	64	+	68	+	68	+	69	+	65
Glucose 1.0%.....	+	65	+	70	+	85	+	85	+	58	+	50
Glucose 2.0%.....	+	80	+	80	+	80	+	95	+	55	+	59
Starch 1.0%.....	+	73	+	58	+	76	+	67	+	48	+	52
Sucrose 0.3%.....				80								
Sucrose 0.15%.....	+	75	+	55	+	75	+	60	+	45	+	42
Peptone 2.0%.....												
Holly Extract.....	+	55	+	58	+	52	+	69	+	50	+	48
Pea Extract.....	-		-		-		-		-		-	
Knop's Solution...	-		-		-		-		-		-	

Germination given in approximate percentage. Time given in minutes.  
The temperature on Sept. 21, 22 and 29 was 18° C.

germination was found in Pea Extract or in Knop's solution. In peptone the formation of bacteria made the estimation of the percentage germination difficult to determine, though there is no doubt that some of the spores liberated their contents within an hour. The temperature of the light culture was 18° C. and those in darkness were maintained at the same temperature.

The spores which were sown on Sept. 22 were obtained from near Sevenoaks, Kent, the previous June; those used on Sept. 29 were from an old stock which had also been collected near Sevenoaks; the date of this collection is not known but they were at least five years old. It is very interesting to note that there was no loss in the vitality of the spores even after five years. This is in accordance with previously observed data, several old collections of the spores of *Reticularia Lycoperdon* have been kept in the Department and they appear to keep their vitality up to about seven years after collecting.

The differences in the rate of germination in the three types of physiological conditions are insufficient to justify any definite conclusions being drawn from them. On the whole the rate seems to be slightly accelerated by heat, but the difference in time

TABLE 2  
*Stemonitis splendens* var. *flaccida*

Solutions used.	Light.				Dark.				Dark and Heat.			
	Sept. 11		Sept. 15		Sept. 11		Sept. 15		Sept. 11		Sept. 15	
	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.
Water.....	—		1	21	—		5	26	—		2	21
Glucose 1.0%.....	1	21	10	26	5	27	15	26	—		—	
Glucose 2.0%.....	—		10	26	50	27	50	26	—		10	26
Starch 1.0%.....	5	27	1	26	5	21	—		15	27	10	26
Sucrose 0.3%.....	—		—		—		—		—		—	
Sucrose 0.15%.....	—		—		20	21	2	20	4	27	1	26
Peptone 2.0%.....	20	20	*		60	26	*		—		*	
Holly Extract.....	—		50	21	—		50	21	—		25	21
Pea Extract.....	—		25	21	—		10	21	—		10	21
Knop's Solution...	15	20	10	20	30	21	5	21	2	27	—	—

Germination given in approximate percentage. Time given in hours.

The temperature on Sept. 11, was 17° C. and on Sept. 15, 18° C.

Contaminated with bacteria.

is insufficient to be of fundamental importance. The most rapid germination was in dilute sucrose solution, where it took place in 42 minutes.

Table 2 shows the results obtained with *Stemonitis splendens* var. *flaccida*. These spores germinated very much more slowly than those of *Reticularia Lycoperdon*. It was also found possible to give the germination as a percentage. The result on the two occasions when the spores were sown is variable but in general it shows that the best results can be obtained with 2.0 per cent glucose, 1.0 per cent starch and with 0.15 per cent sucrose, it is interesting to note that no germination was obtained with the stronger solution of sucrose. Knop's solution gave satisfactory results except in the dark and heat. Extracts of pea and holly proved to be very suitable as was to be expected from the habitat where this species is to be found. The result with 2.0 per cent peptone was interesting, in both light and darkness good germination was generally obtained, but under the influence of heat no swarm spores were obtained. In the second attempt the formation of bacteria in this solution prevented any observations being made. The general inference from this table is that the spores of *Stemonitis splendens* var. *flaccida* germinate better in the dark



TABLE 3

*Fuligo septica*

Solutions used.	Light.				Dark.				Dark and Heat.			
	Sept. 14		Sept. 15		Sept. 14		Sept. 15		Sept. 14		Sept. 15	
	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.
Water . . . . .	—		—		—		—		—		+	23
Glucose 1.0% . . . . .	—		+	24	—		+	25	—		+	25
Glucose 2.0% . . . . .	—		+	24	—		+	24	+	24	—	
Starch 1.0% . . . . .	—		—		—		—		—		—	
Sucrose 0.3% . . . . .	—		—		—		—		—		—	
Sucrose 0.15% . . . . .	—		—		—		—		—		—	
Peptone 2.0% . . . . .	—		—		—		—		—		—	
Holly Extract . . . . .	—		—		—		—		—		—	
Pea Extract . . . . .	—		—		—		—		—		—	
Knop's Solution . . . . .	—		—		—		—		—		—	

Germination given without percentage, but in no case did it exceed 10 per cent of the spores examined. Time given in hours.

The temperature on both Sept. 14 and 15 was 18° C.

than in the light and that the effect of heat is to decrease the rate of germination in all solutions except starch.

The sporangia used in these two cases were obtained from different parts of Epping Forest during the autumn of 1927.

Table 3 shows the germination of *Fuligo septica*. It was very much more difficult to obtain any definite results with these spores. The best was with 1.0 per cent glucose, where germination occurred in all three conditions, and in one instance on both occasions when germination was tried. With 2.0 per cent glucose the same result was obtained once in the light, once in the dark and once in darkness and heat. In one case it was obtained in water, in darkness and heat. No other solution gave any positive results at all. *Fuligo septica* grows chiefly on rotten wood and it is remarkable that no swarm spores were found in extracts of natural substances. The spores used in these experiments were collected from diverse localities, those used on Sept. 14 from Oxshott, Surrey, those on Sept. 15 from near Bournemouth, Hampshire.

Table 4 shows the germination of *Lycogola epidendrum*. Although this species grows and fruits generally throughout the

TABLE 4

*Lycogola epidendrum*

	Light.		Heat.		Heat and Light.	
	Sept. 23		Sept. 23		Sept. 23	
	Germ.	Time.	Germ.	Time.	Germ.	Time.
Water.....	2	68	4	72	—	69
Glucose 1.0%.....	15	68	70	68	—	
Glucose 2.0%.....	10	69	10	69	—	
Starch 1.0%.....	15	69	8	69	3	
Sucrose 0.3%.....	—	—	—	—	—	
Sucrose 0.15%.....	8	71	35	71	—	
Peptone 2.0%.....	—	—	—	—	—	69
Holly Extract.....	—	—	50	71	—	
Pea Extract.....	—	—	—	—	—	
Knop's solution.....	—	—	—	—	—	

Germination given in approximate percentage. Time in hours. The temperature was 18° C.

year, scarcely any swarm spores were produced in the heat, though in glucose, starch and sucrose fair germination was found in both light and darkness. No swarm spores were found in Knop solution or in 0.3 per cent sucrose. The germination in tap water was not so good as in the other solutions. The time taken for any swarm spores to appear was considerably longer than in any of the species already considered, but the percentage germination was quite good. The material was collected from Oxshott, Surrey in July 1927, and the results are in agreement with the preliminary observations made.

Table 5 gives the results of the germination of *Trichia varia*. It is generally found very difficult to obtain swarm spores from any species of this genus. This may in part be due to the time which must elapse between the sowing of the spores and the appearance of the swarm spores. It was not found possible to examine the cultures sufficiently often to give the times of germination in hours and they are therefore represented in days. The most rapid germination was five days. Germination was most successful in 1.0 per cent starch, with this swarm spores were obtained on all occasions. There was a general tendency for less germination in heat than in the colder conditions. Those grown in light on Jan. 11 were placed in an unheated greenhouse,

TABLE 5

*Trichia varia*

Solutions Used.	Light.				Dark.				Dark and Heat.			
	Dec. 14.		Jan. 11		Dec. 14		Jan. 11		Dec. 14		Jan. 11	
	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.	Germ.	Time.
Water .....	2	7	2	6	1	6	5	6	—	—	—	—
Glucose 2.0% .....	1	7	4	6	3	6	1	5	3	6	—	—
Glucose 1.0% .....	2	7	1	5	25	5	1	6	1	6	—	—
Starch 1.0% .....	2	7	5	5	2	6	1	5	2	6	2	6
Sucrose 0.3% .....	—	—	1	6	—	—	—	—	—	—	—	—
Sucrose 0.15% .....	—	—	—	—	—	—	—	—	1	6	—	—
Peptone 2.0% .....	—	—	—	—	1	6	—	—	1	6	—	—
Holly Extract .....	—	—	1	6	2	5	—	—	—	—	—	—
Pea Extract .....	—	—	—	—	—	—	—	—	—	—	—	—
Knop's Solution .....	—	—	2	6	1	5	1	5	—	—	—	—

Germination given in approximate percentage. Time given in days. The temperature on Dec. 14 varied considerably, being as low as  $-2^{\circ}$  C. on at least one occasion, that on Jan. 11 was  $18^{\circ}$  C.

and it is known that one night the temperature fell to  $30^{\circ}$  F., yet the difference in the rate of germination from that on Dec. 14 when the cultures were set up in the laboratory at  $18^{\circ}$  C. was not very noticeable, in general there appeared to be a slight slowing off of the rate, though germination occurred in some cultures only at the lower temperature. *Trichia varia* is a common winter species and it was to be expected that a low temperature would not impede germination. 25 per cent germination in glucose on Dec. 14 was not in accordance with what was found in any of the other cultures and stands apart as a much better proportion of viable swarm spores than anywhere else, that this could not be repeated suggests that these spores were in some way different from the rest, although they were obtained from the same mass of sporangia as the others. All the spores used were collected in Epping Forest during October 1927.

From these five tables it can be seen that the spores of very diverse types of Mycetozoa are capable of being germinated in artificial solutions with comparative certainty though the quantity of germination will vary within wide limits. The time taken for germination varies very much in the different genera though

in general species of the same genus germinate in approximately the same time in the same solution. The growth in light, darkness and heat seems to affect species in different ways though in general heat is not advantageous to growth of the swarm spores. Light is of advantage to some while darkness benefits others, the habitat on which they live in nature does not seem to influence these results.

Attempts were made to try and grow some of these forms on agar plates, but, although they germinated readily in solutions, no positive results were obtained with agar. This is probably at any rate partly due to the comparative dryness of the agar plates and also to the facility with which other fungal spores start to grow and soon cover the plates. No satisfactory way of preventing the appearance of these fungi was found. The addition of acid or alkalis to the medium was thought to have a deleterious effect upon the swarm spores and was not adopted.

#### DISCUSSION

Comparatively little has been published on the germination of Mycetozoa under artificial conditions. In 1894 Durand (3) studied the germination of *Enteridium Rozeanum*. He found that the spores required a period of rest before germination would take place. The best temperature to obtain a large proportion of swarm spores he concluded was about 28° C. We found that satisfactory germination was obtainable at about 18° C. Durand observed that in some cases swarm spores were produced having a flagellum at each end.

Previously de Bary (1) had succeeded in germinating the spores of *Fuligo septica* and *Trichia varia*, and observed that occasionally swarm spores with two flagella were produced, though in this case, it is doubtful whether both were formed at the same end.

In 1893 McClatchie (6) reported the germination of the spores of *Reticularia Lycoperdon*. He found that no period of rest was required, and that it was possible to induce the formation of swarm spores within an hour after placing them in distilled water. He also succeeded in germinating the spores of *Diachaea leucopoda*, *Hemitrichia Vesparium*, *Fuligo septica* and *Badhamia capsulifera*; only a small proportion of these produced swarm spores, and he

concluded that this was due to the fact that the collections were two months old.

Miller (7) in 1899 carried out further experiments on the germination of Mycetoza spores under aseptic conditions. He cultivated these in flasks using infusions of hay. In some cases he was able to obtain not only plasmodia but also fructifications in his cultures.

In 1906 Constantineanu (2) published his important contribution to the subject. He studied the germination of a large number of species both in organic and in inorganic substances, and endeavoured to discover what substances were made use of by the swarm spore during its growth. It is interesting to compare the time taken for germination in the species he examined with our own. In distilled water he found that the rate of germination varied from 30 minutes in *Reticularia Lycoperdon* to 24 hours in *Physarum didermoides*, though in the latter species he admits that sometimes they took as long as two or three days, or in a few cases even longer. He tried the effect of carbohydrates, but came to the conclusion that these alone were insufficient to give good results; he found that sucrose was better than glucose. He also made experiments with natural substances, such as extracts of bark, branches and seeds, he found that these were very suitable for the germination of all species of Mycetoza. He also went on to study the growth of the plasmodia in various media.

In general, his results are in agreement with our own. In *Reticularia Lycoperdon* he obtained germination in 30 minutes in water, while we found that it took from 64 to 82 minutes, but he found that in order to obtain a 50 per cent formation of swarm spores the time required was from 2 to 3 hours, while in the present instance a 50 per cent germination was obtainable in little over an hour. McClatchie also found that the swarm spores of this species appeared in about an hour after sowing.

In *Stemonitis splendens* var. *flaccida* he obtained germination in from 5 to 6 hours; in the present case the time was considerably longer, from about 21 hours. To get a 25 per cent germination took up to from 12 to 48 hours, whereas we found in a solution of peptone a 60 per cent germination in 26 hours, and in extracts of Holly in 21 hours.

*Fuligo septica* we found very difficult to germinate at all, Constantineanu found swarm spores in from 30 minutes to 90 minutes. We found no germination in water, except in heat and darkness; germination was best in glucose solutions.

*Lycogola epidendrum* he germinated in 60 hours in water; those we tried took from 68 to 72 hours, though the percentage of germination was much lower. Only in Holly extract did the percentage germination reach anything comparable with what Constantineanu obtained.

He found that *Arcyria denudata* germinated in about 9 hours, while we found that in the related genus *Trichia*, *T. varia* required as much as five days.

In most cultures the swarm spores appeared to be quite normal; they remained active for a varying period, sometimes becoming amoeboid, and then regaining their flagellated condition. Some of the cultures were kept for several days, but in no case did any indications of the formation of a plasmodium appear. It is probable that they required some substratum on which to grow, and failing this they were incapable of further life. It has been observed that plasmodia of various species can be produced in agar cultures, particularly when grown in tubes, in this case the plasmodium generally migrates from the surface of the agar and swarms up the surface of the glass.

In 1907 Pinoy (8) suggested that bacteria played a part in the germination of the spores of all Mycetozoa. Although in the older cultures these are almost invariably present, yet in species like *Reticularia Lycoperdon* where germination is rapid no bacteria could be detected at the time when the swarm spores appeared, though later the cultures became contaminated with them.

Since the appearance of Constantineanu's paper little has been published on the subject, though a few workers have obtained plasmodia artificially for various purposes. In 1927 Gilbert (4) suggested that those bodies appearing in cultures of swarm spores possessing more than one flagellum were foreign bodies. He found both in *Hemitrichia clavata* and in *Stemonitis fusca* organisms having either a flagellum at each end or two at the anterior. He considered that these were Protozoa, and referred them to the genus *Cercomonas*, as *Cercomonas longicauda*. In our cultures of

*Stemonitis splendens* var. *flaccida* we have observed forms with as many as three flagella. Though they appear sparingly in some culture solutions they are entirely absent in others. No multi-flagellated swarm spores were found in any of the other species examined.

Multiflagellated swarm spores have been sometimes observed in cultures of a number of species, and it seems unlikely that these are all due to the presence of a Protozoan invader. It is not unreasonable to assume that under certain abnormal nutrient conditions swarm spores with more than a single flagellum can be produced.

#### SUMMARY

1. Spores of several species of Mycetozoa including *Reticularia Lycoperdon*, *Fuligo spetica*, *Stemonitis splendens* var. *flaccida*, *Lycogola epidendrum* and *Trichia varia*, have been successfully germinated in artificial solutions.

2. The rate of germination varied in the different species; *Reticularia Lycoperdon* taking about an hour, *Trichia varia* about five days.

3. Tables are given showing the proportion of germination and the time taken, in respect to a number of organic substances. The effect of light, darkness and combined darkness and heat was tried. The results differ in the various species and no general conclusions could be arrived at.

4. Evidence is brought forward to show that the length of time taken by the spores of a species collected from different localities to germinate does not differ appreciably, no more than do the spores of the same mass of sporangia when grown at different times under the same conditions. It has been found that the spores of *Reticularia Lycoperdon* do not lose their vitality in five years, though after seven years germination is uncertain. The same vitality is not shown by other species, *Trichia varia* losing its power of germination in about a year.

Our thanks are due to Professor R. R. Gates for reading through the proofs and for helpful criticisms during the work.

KING'S COLLEGE,  
UNIVERSITY OF LONDON,  
February, 1928.

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## NOTES AND BRIEF ARTICLES

### A Correction

In the May-June issue of MYCOLOGIA, 1928, volume 20, number 3, page 153, was published a description of a new genus of fungi. Line 13 from the top of page 153, reading "*Monilinia fruticola* (Winter) comb. nov." should be deleted. EDWIN E. HONEY.

### ROMUALDO GONZALEZ FRAGOSO

† JUNE 3, 1928

With the death of Doctor Romualdo González Fragoso, chief of the Cryptogamic Laboratory of the Real Jardín Botánico de Madrid, Spanish mycology has lost its most distinguished scholar. In a short period of time, the Spanish-speaking world has lost its two most eminent mycologists: the well-known Doctor Carlos Spegazzini, an Italian by birth, and Professor Gonzalez Fragoso.

The work he leaves behind is of the greatest importance, especially along the lines of systematic mycology. Our present knowledge of the fungous flora of Spain and Portugal should justly be credited, almost exclusively, to his tireless efforts. In late years he realized the convenience of bringing together in monographic form his numerous works on the flora of the Iberian peninsula and had already published two volumes on the Uredinales, one on the Hyphales and a complete census of the other. Deuteromycetes, exclusive of the Sphaeropsidales, and also of the Ustilaginales. He has also published contributions on the fungi of Morocco, Mexico and Santo Domingo. Of this latter country, he was making an extensive study, and had published, in collaboration, fifteen serial contributions. His untimely death came just as he was completing his sixteenth series.

CARLOS E. CHARDON AND R. CIFERRI.

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